US011366097B2

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Okada et al.

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(45) **Date of Patent: Jun. 21, 2022**

(54) **CELL IMAGING METHOD, CELL IMAGING APPARATUS, PARTICLE IMAGING METHOD, AND PARTICLE IMAGING APPARATUS**

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(73) Assignee: **SYSMEX CORPORATION**, Hyogo (JP)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **15/883,329**

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(30) **Foreign Application Priority Data**

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(51) **Int. Cl.**
G01N 33/49 (2006.01)
G01N 15/14 (2006.01)
(Continued)

(52) **U.S. Cl.**
CPC ***G01N 33/4915*** (2013.01); ***C12Q 1/68*** (2013.01); ***G01N 1/10*** (2013.01); ***G01N 15/1404*** (2013.01); ***G01N 15/147*** (2013.01); ***G01N 15/1436*** (2013.01); ***G01N 21/17*** (2013.01); ***G01N 33/48728*** (2013.01); ***G01N 33/5044*** (2013.01);
(Continued)

(58) **Field of Classification Search**
CPC G02B 21/004; G02B 21/33; A61B 1/00; G01N 33/4915; G01N 21/17; G01N 33/48728; G01N 33/5044; G01N 2015/1006; G01N 2015/144; G01N 2015/1454; C12Q 1/68
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

8,935,107 B1 * 1/2015 Adamovsky G01F 1/74 702/50
2003/0133096 A1 * 7/2003 Aroussi A61B 5/027 356/28

(Continued)

FOREIGN PATENT DOCUMENTS

CN 104407436 A 3/2015
JP 2008-215999 A 9/2008

(Continued)

OTHER PUBLICATIONS

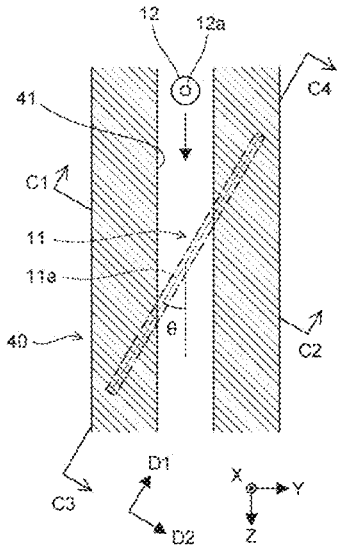
The Japanese Office Action dated Apr. 13, 2021 in a counterpart Japanese patent application No. 2017-147660.

Primary Examiner — Jefferey F Harold
Assistant Examiner — Timothy R Newlin
(74) *Attorney, Agent, or Firm* — Crowell & Moring LLP

(57) **ABSTRACT**

Disclosed is a cell imaging method including: forming a light sheet with respect to a flow cell; causing a measurement sample containing a plurality of cells to flow in the flow cell; and receiving lights generated from the plurality of cells passing through the light sheet, by an imaging device via an element configured to extend a depth of focus, and taking images of the plurality of cells by the imaging device.

15 Claims, 20 Drawing Sheets



(51) **Int. Cl.**

G01N 1/10	(2006.01)
C12Q 1/68	(2018.01)
G01N 33/487	(2006.01)
G01N 33/50	(2006.01)
G01N 21/17	(2006.01)
G01N 15/10	(2006.01)

(52) **U.S. Cl.**

CPC *G01N 2015/1006* (2013.01); *G01N 2015/144* (2013.01); *G01N 2015/1454* (2013.01)

(56) **References Cited**

U.S. PATENT DOCUMENTS

2004/0104681	A1*	6/2004	Mitrovic	G01N 15/0227 315/111.21
2009/0231660	A1*	9/2009	Huang	G02B 26/125 359/226.1
2010/0278400	A1*	11/2010	Piestun	G01N 21/6456 382/128
2011/0249866	A1*	10/2011	Piestun	H04N 13/204 382/103
2011/0310226	A1*	12/2011	McEldowney	G01B 11/2513 348/46
2013/0147925	A1*	6/2013	Lew	H04N 13/204 348/49
2013/0302905	A1*	11/2013	Kalkbrenner	G01N 21/6428 436/172
2014/0099659	A1*	4/2014	Keller	G01N 21/6486 435/29
2014/0239073	A1	8/2014	Toyoda et al.	
2014/0254005	A1*	9/2014	Lippert	G02B 21/367 359/385
2014/0340483	A1*	11/2014	Ritter	G01N 21/6458 348/46
2014/0346328	A1*	11/2014	Niu	G02B 5/1842 250/225
2014/0353522	A1	12/2014	Wu et al.	
2015/0022881	A1	1/2015	Loza Alvarez et al.	
2015/0177065	A1*	6/2015	Wu	G01N 15/0211 356/402
2015/0192510	A1	7/2015	Piestun et al.	
2015/0379695	A1*	12/2015	Naruse	H04N 1/409 348/234
2016/0048963	A1*	2/2016	Piestun	G06K 9/6807 382/154
2016/0125610	A1*	5/2016	Piestun	H04N 13/204 348/46
2016/0327779	A1*	11/2016	Hillman	G02B 21/367
2016/0370570	A1	12/2016	Foelling	
2017/0082531	A1	3/2017	Okada et al.	
2018/0074305	A1*	3/2018	Atzler	G01N 21/6458
2019/0196167	A1*	6/2019	Maddox	G02B 21/0032
2019/0204580	A1*	7/2019	Fahrbach	G02B 21/0032

FOREIGN PATENT DOCUMENTS

JP	2014-164721	A	9/2014
JP	2014-224814	A	12/2014
JP	2016-507078	A	3/2016
JP	2016-525225	A	8/2016
JP	2017-15742	A	1/2017
JP	2017-504836	A	2/2017
JP	2017-58352	A	3/2017
WO	2014/117079	A1	7/2014
WO	2015/109323	A2	7/2015

* cited by examiner

FIG. 1

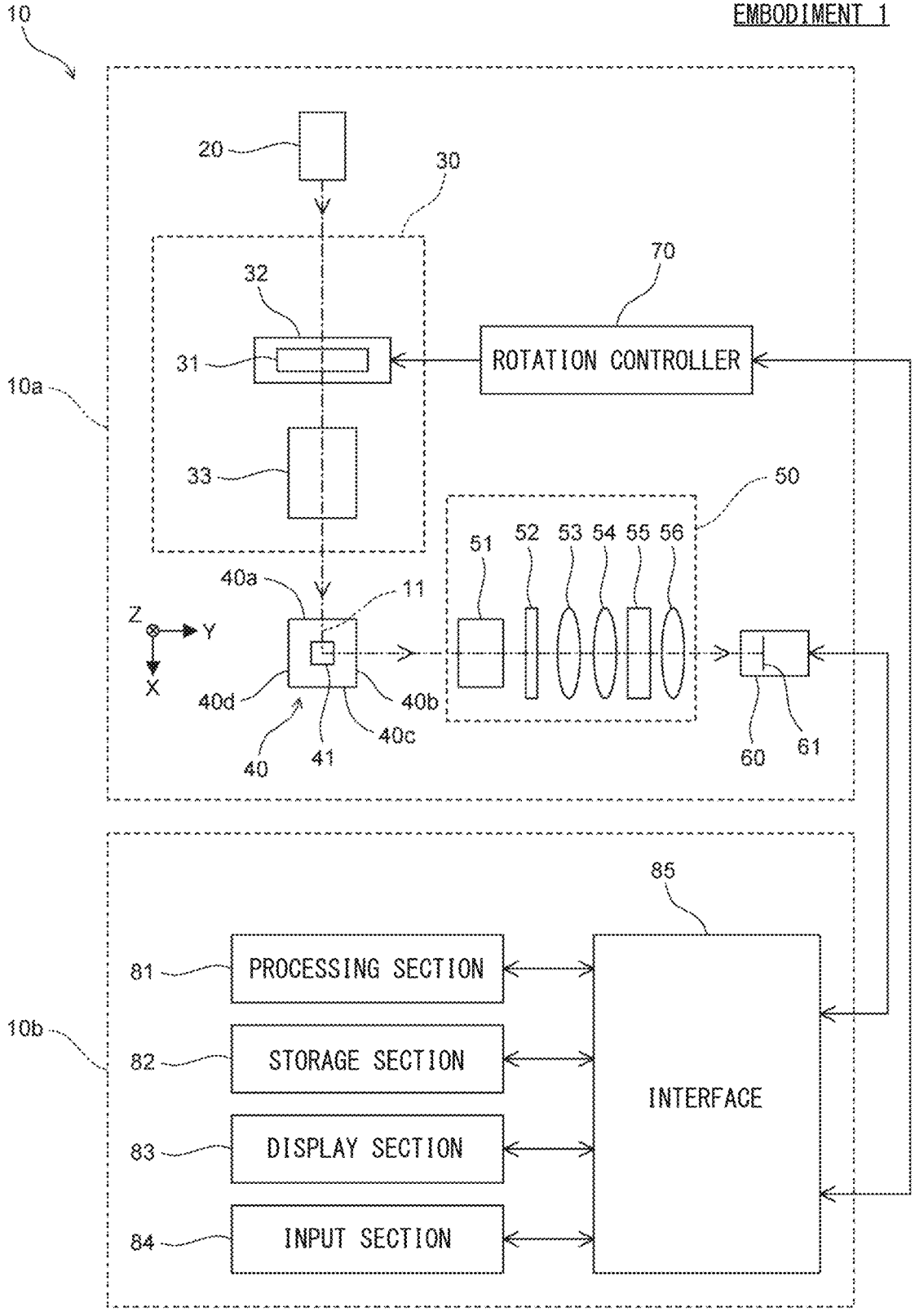

EMBODIMENT 1
10
20
30
32
31
70
ROTATION CONTROLLER
10a
33
50
51
52
53
54
55
56
40a
11
Z
Y
X
40d
40b
40c
40
41
60
61
85
81
PROCESSING SECTION
10b
82
STORAGE SECTION
INTERFACE
83
DISPLAY SECTION
84
INPUT SECTION

FIG. 2A

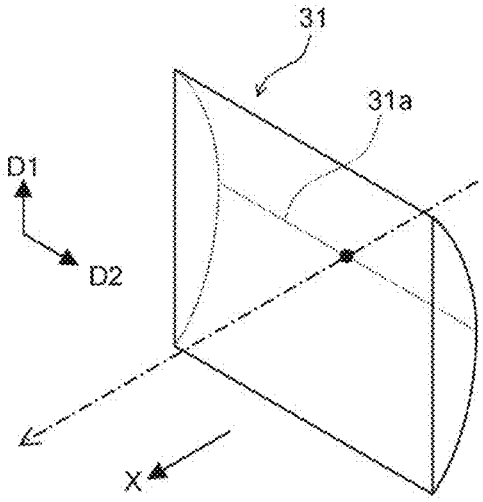


FIG. 2B

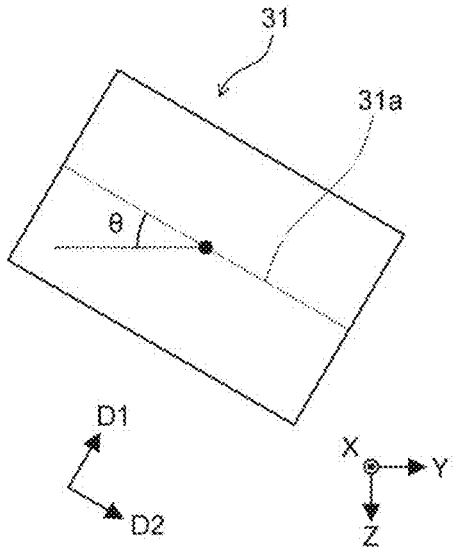


FIG. 2C

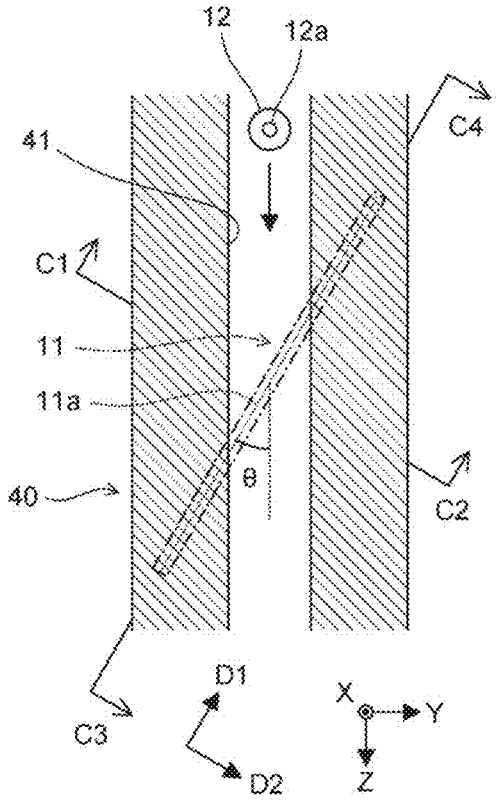


FIG. 2D

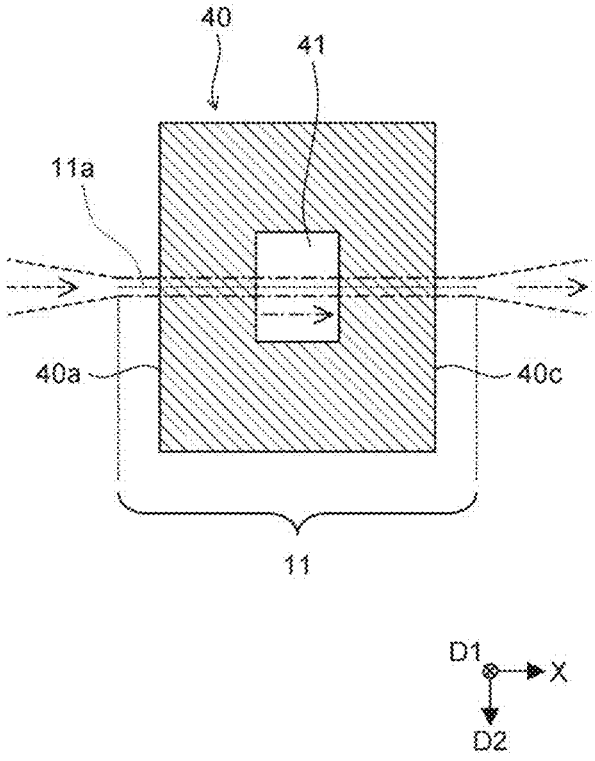


FIG. 3A

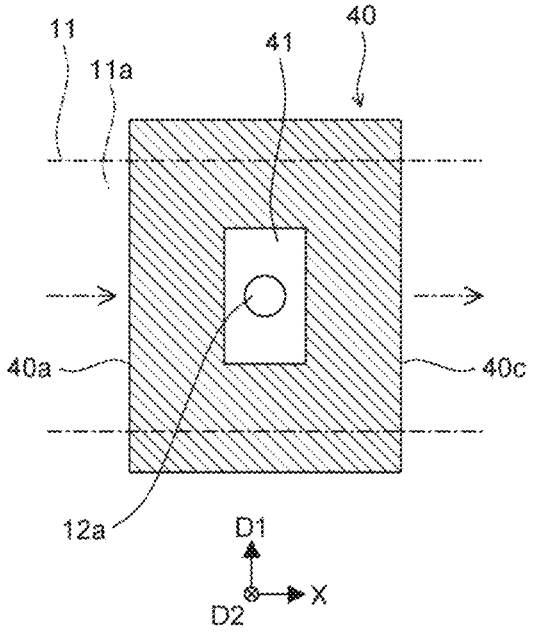


FIG. 3B

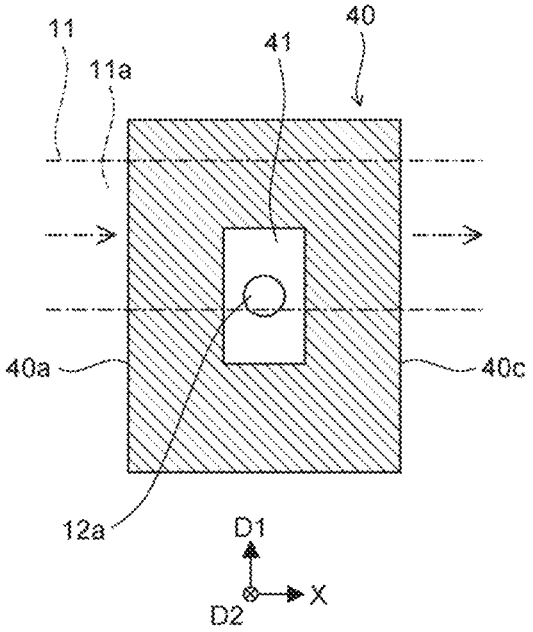


FIG. 3C

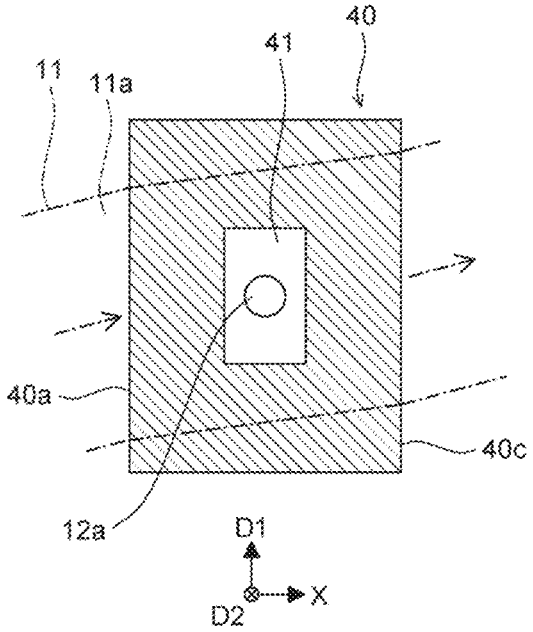


FIG. 3D

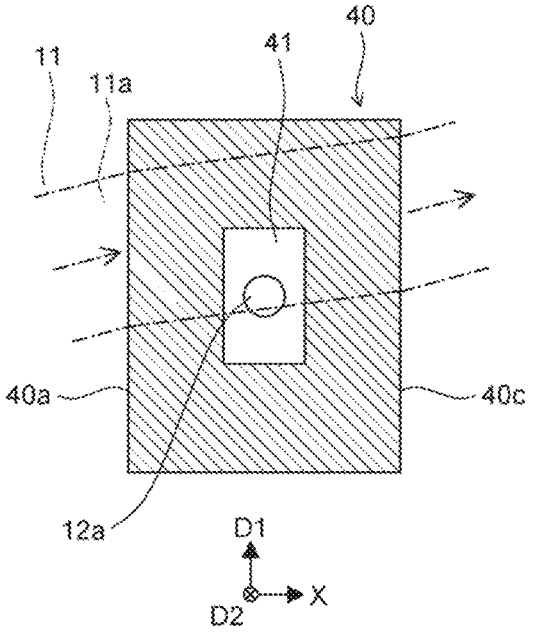


FIG. 4A

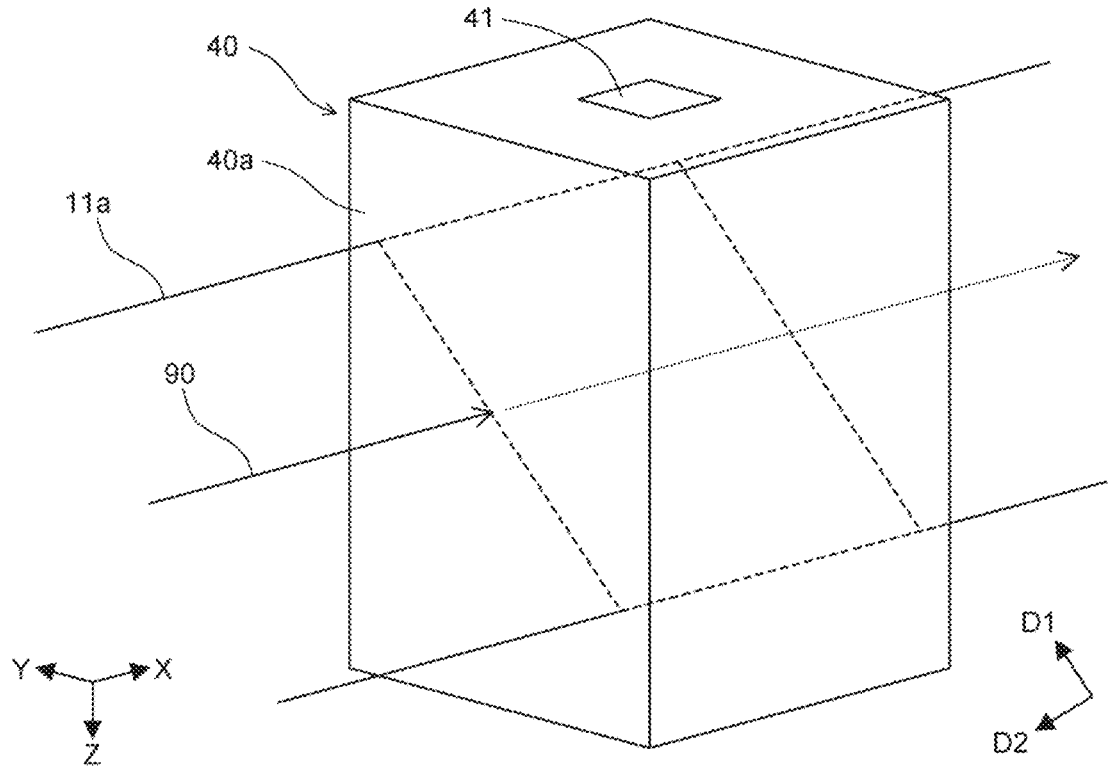


FIG. 4B

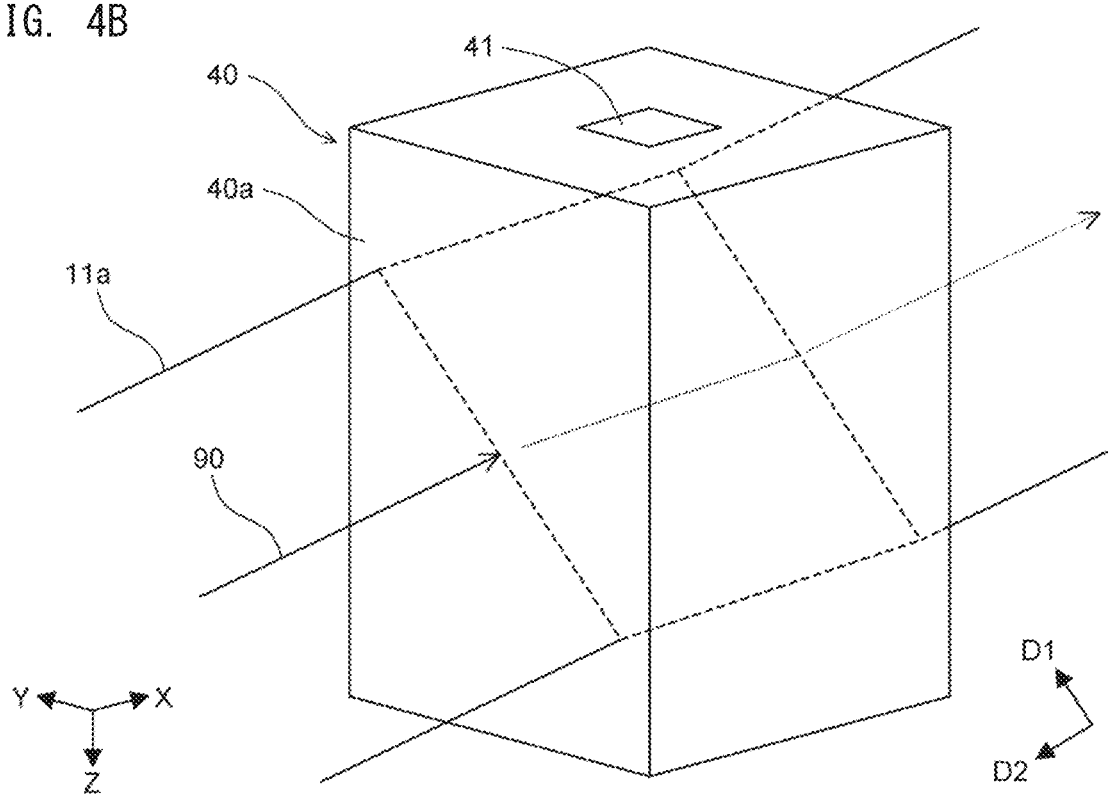


FIG. 5A

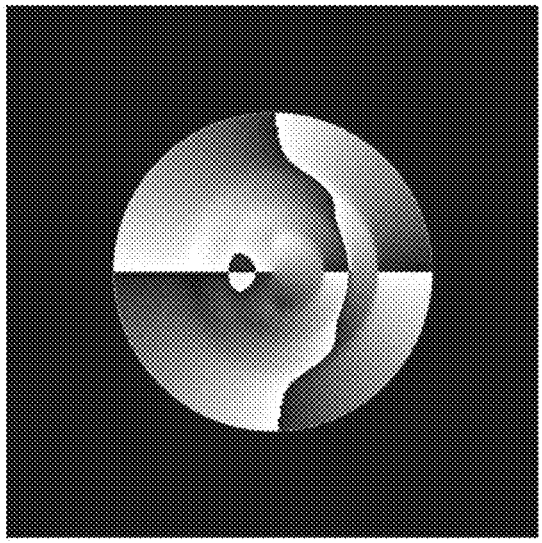


FIG. 5B

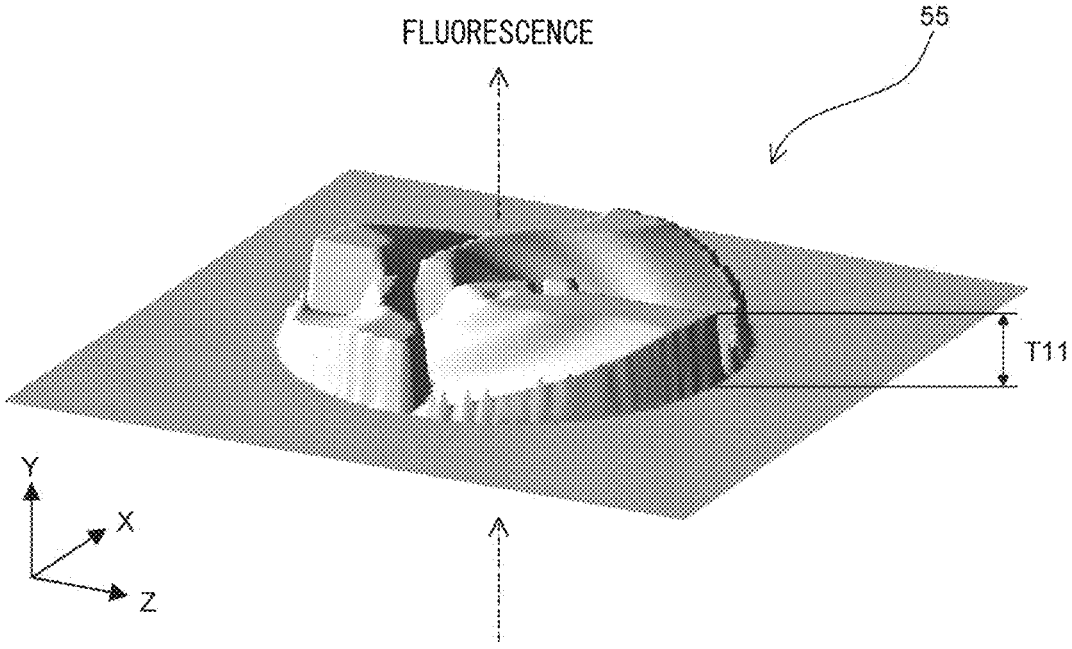


FIG. 6

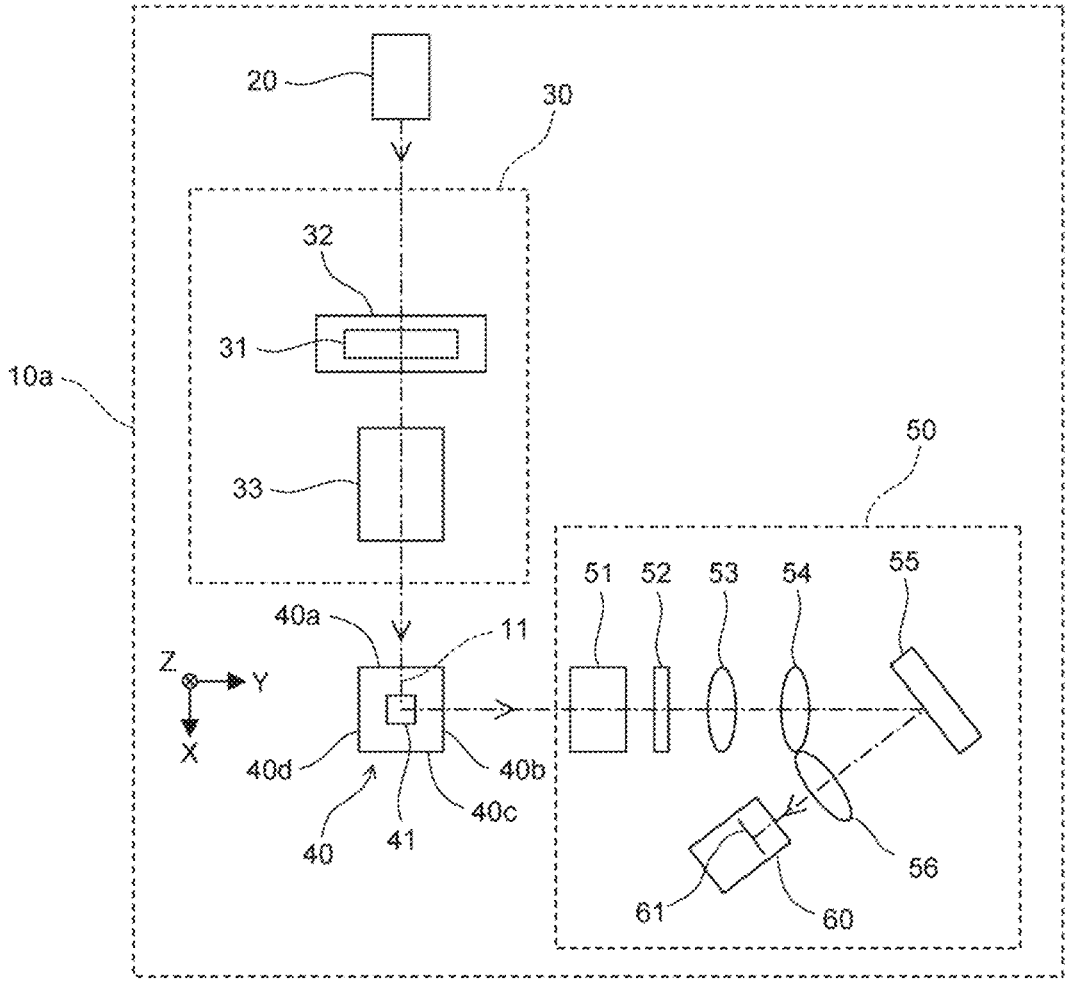

20
30
32
31
10a
33
50
51
52
53
54
55
40a
11
Z
Y
X
40d
40b
40c
40
41
56
61
60

FIG. 7A

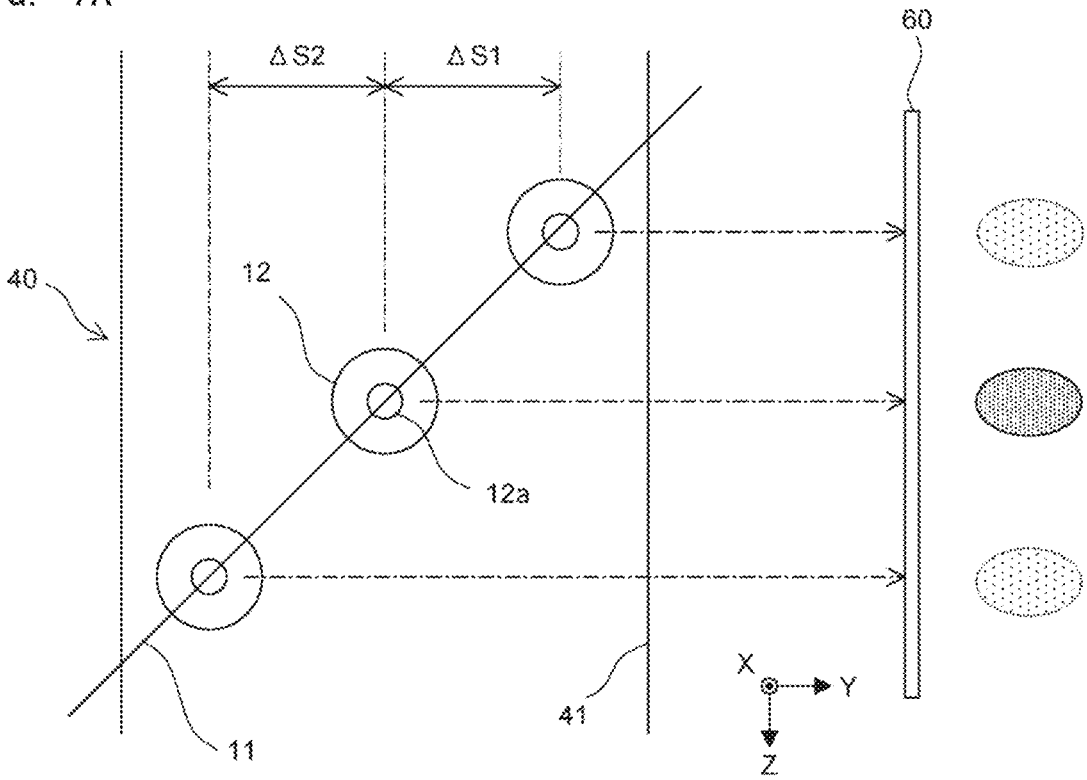


FIG. 7B

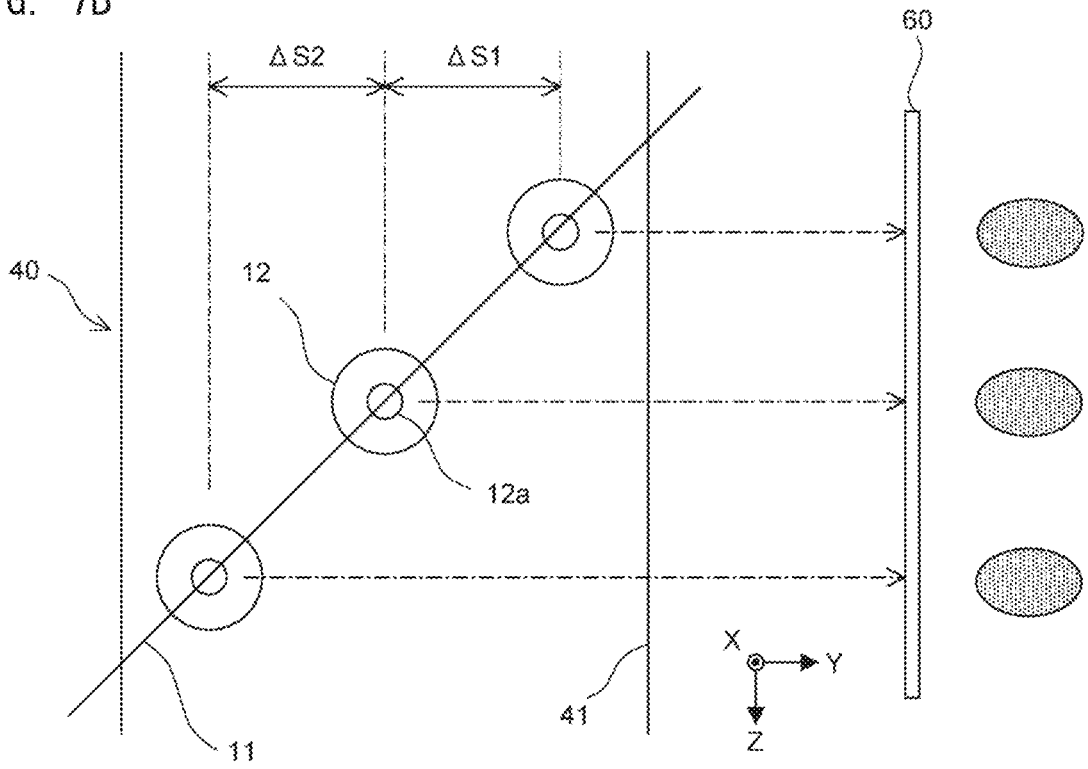


FIG. 8

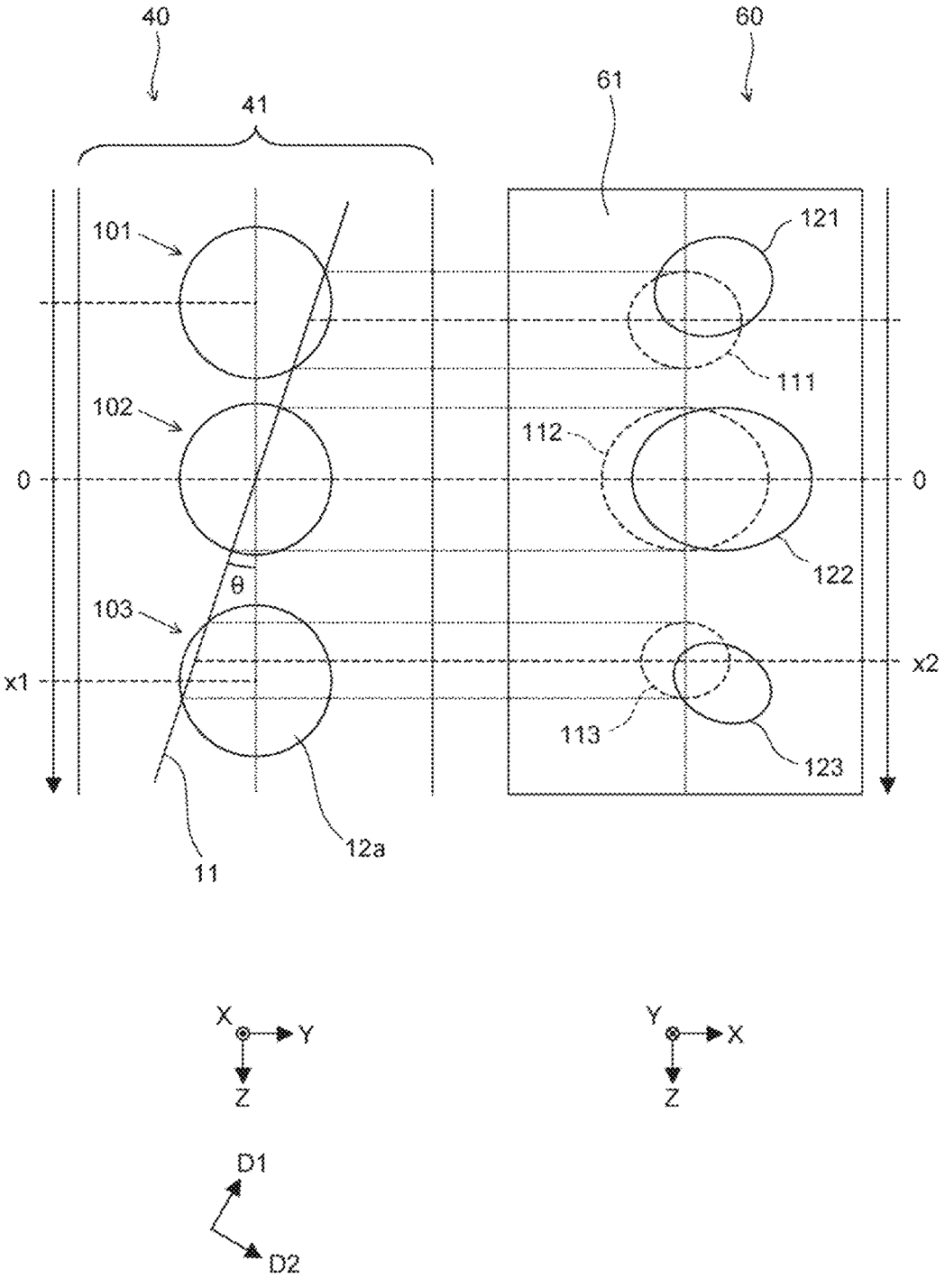

40
60
41
61
101
121
111
102
112
0
0
θ
122
103
x1
x2
113
123
11
12a
X
Y
Z
Y
X
Z
D1
D2

FIG. 9

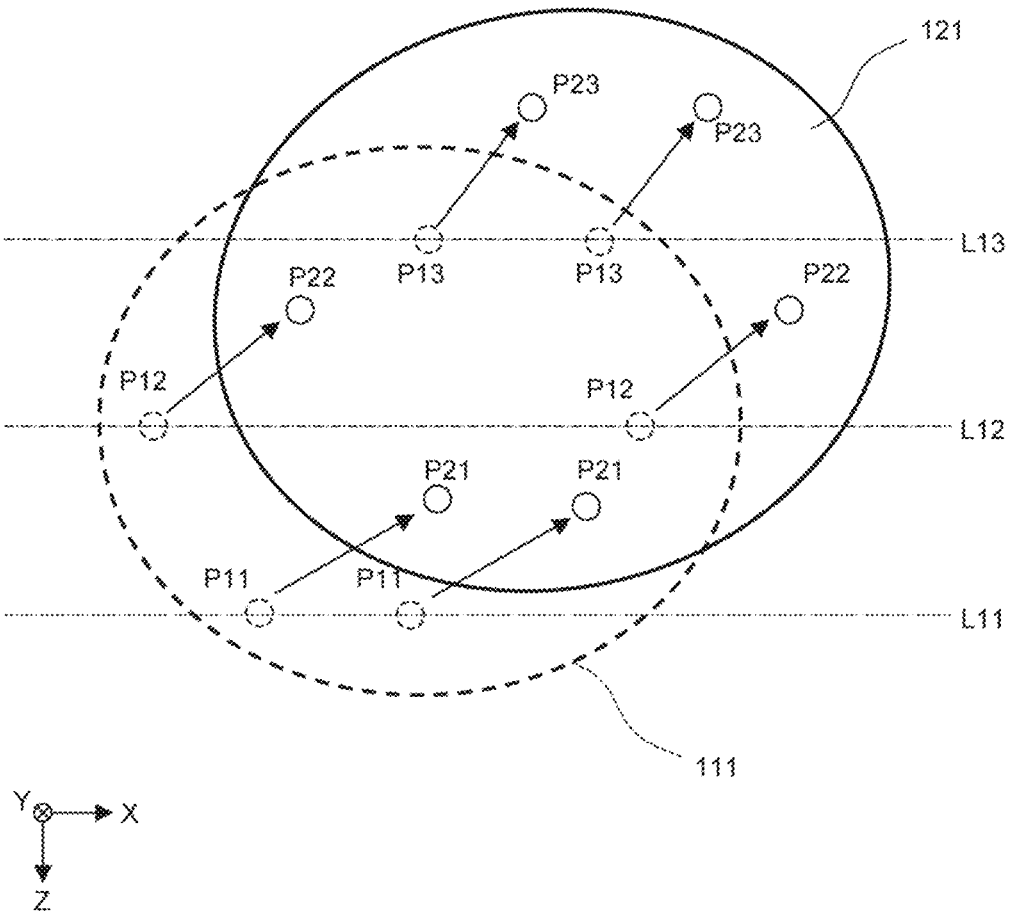

121
P23
P23
L13
P22
P13
P13
P22
P12
P12
L12
P21
P21
P11
P11
L11
111
Y
X
Z

FIG. 10A

FARTHEST POSITION

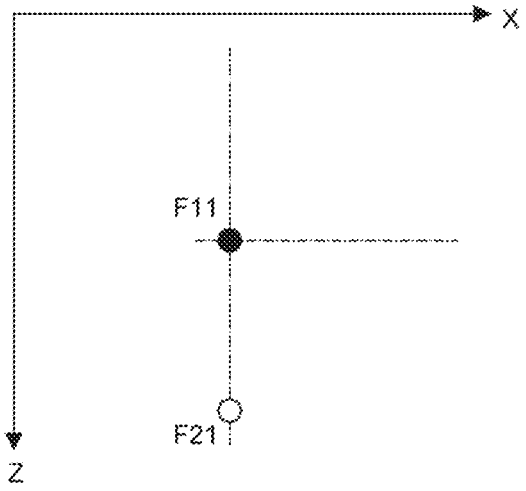


FIG. 10B

INTERMEDIATE POSITION

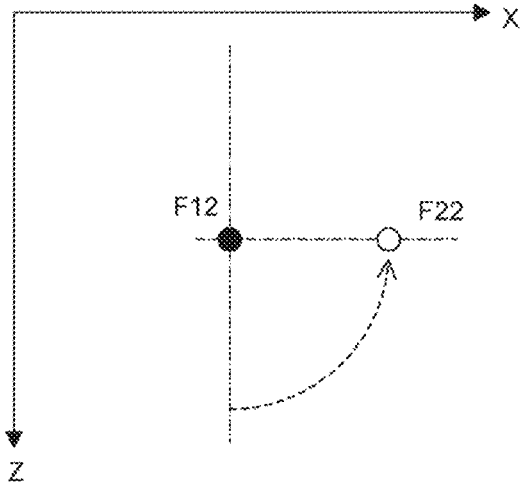


FIG. 10C

CLOSEST POSITION

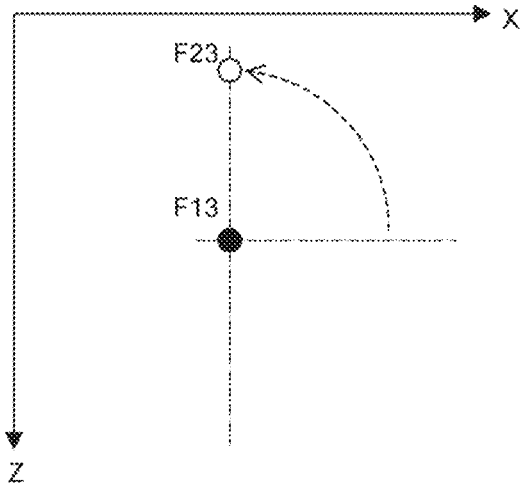


FIG. 11A

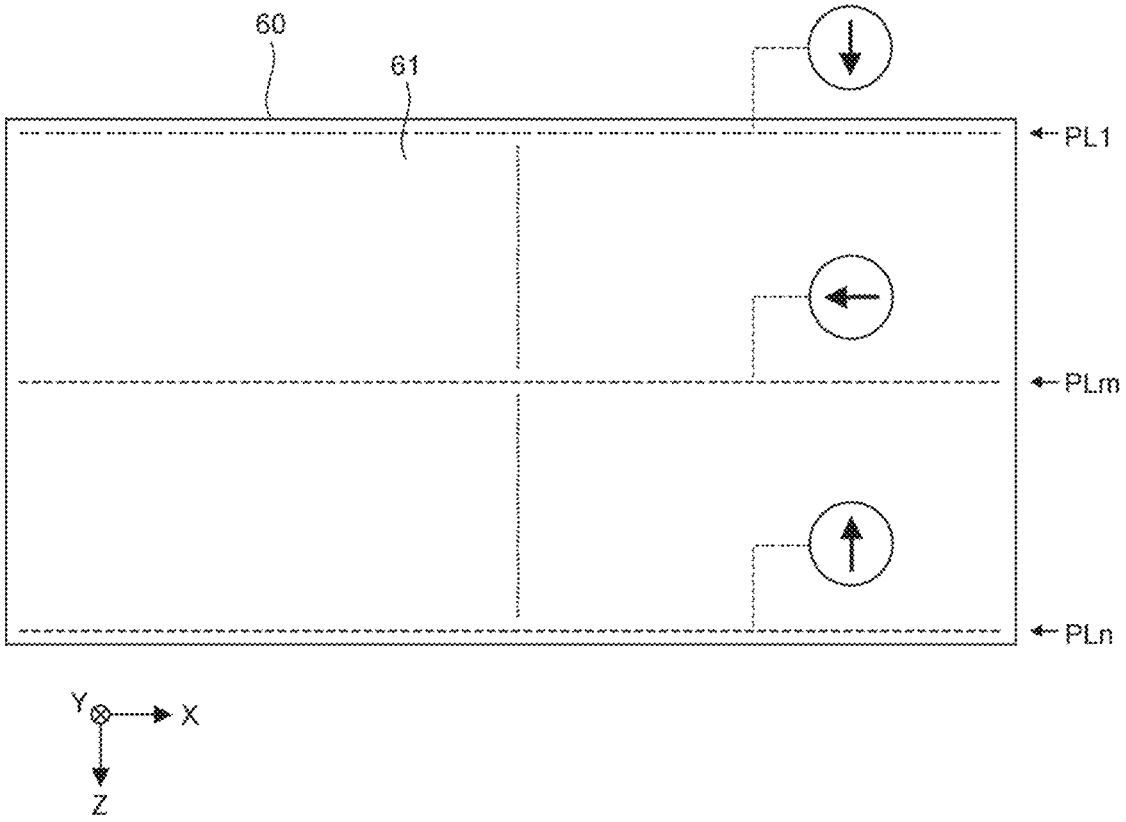


FIG. 11B

PIXEL LINE	X CORRECTION VALUE	Z CORRECTION VALUE
PL1	Xa1	Za1
PL2	Xa2	Za2
⋮	⋮	⋮
PLn	Xan	Zan

FIG. 12A

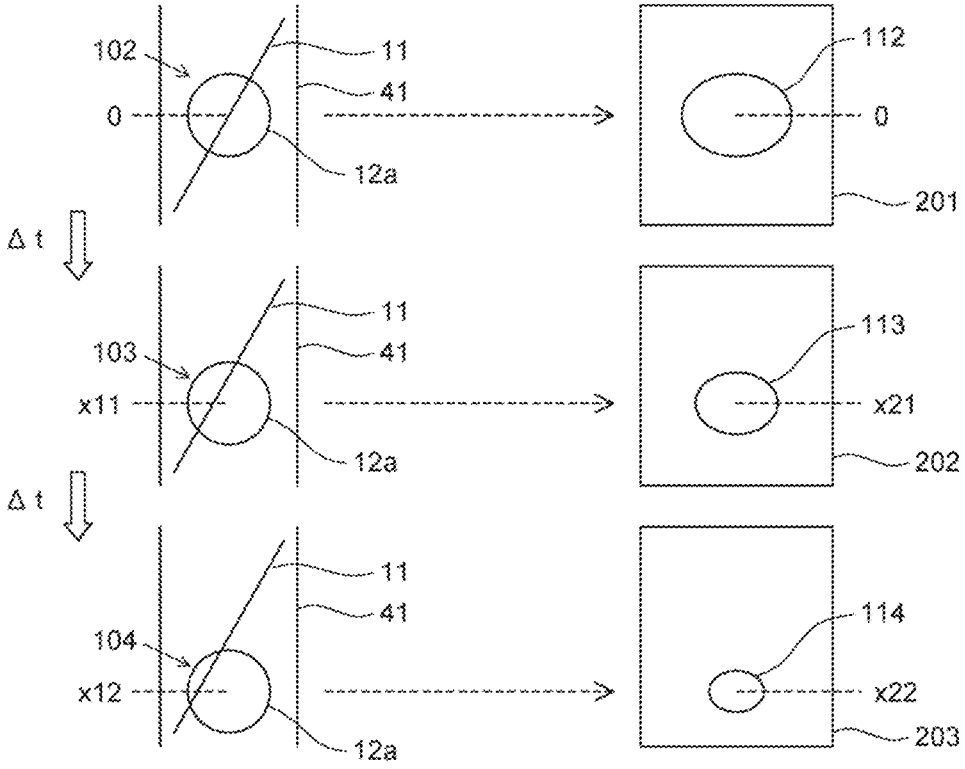


FIG. 12B

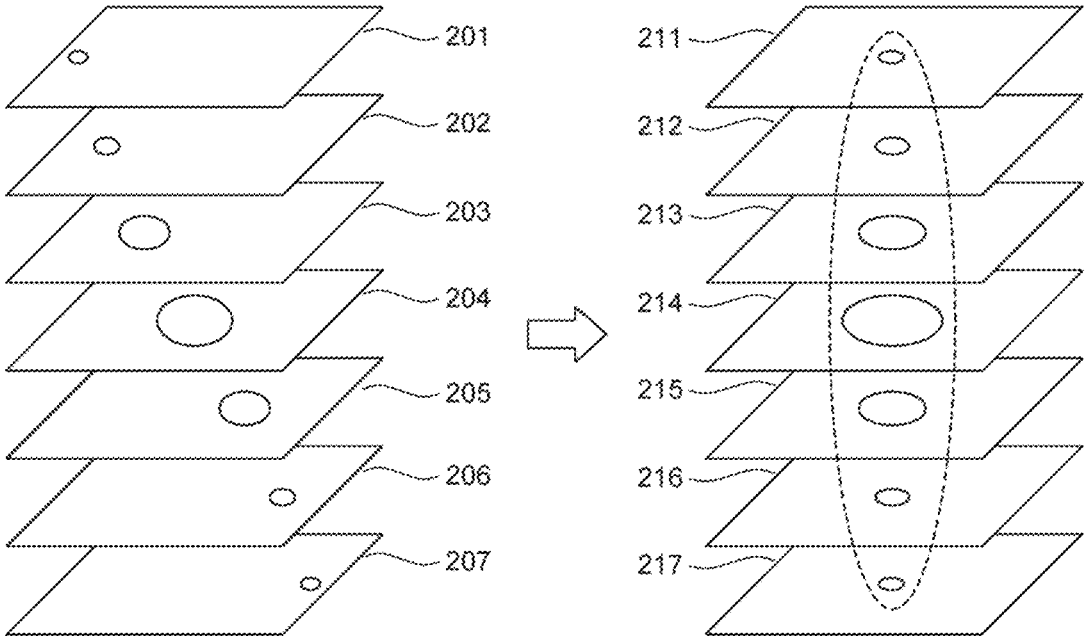


FIG. 13A

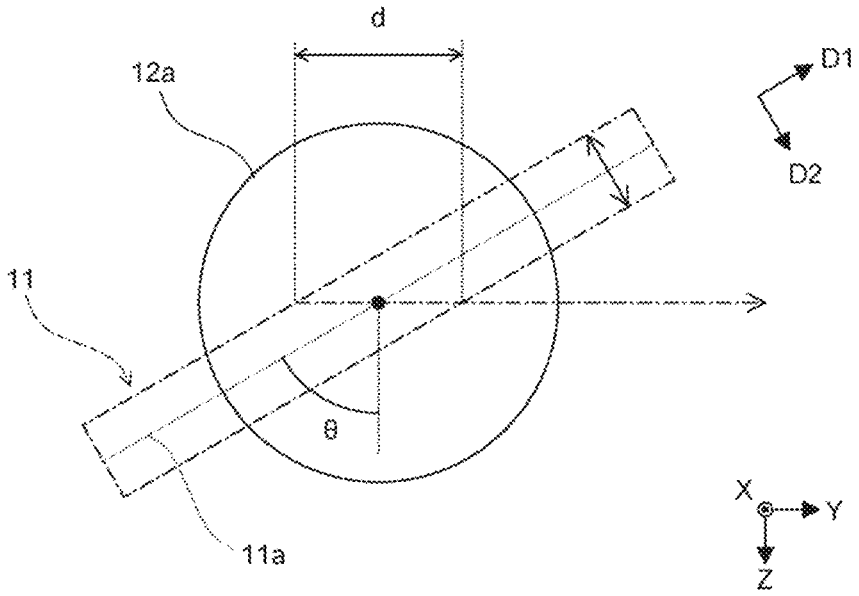


FIG. 13B

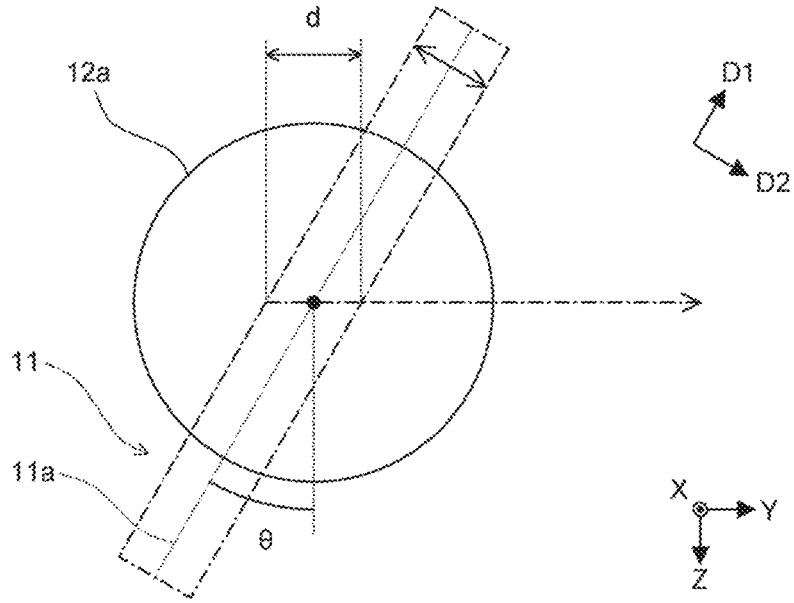


FIG. 14A

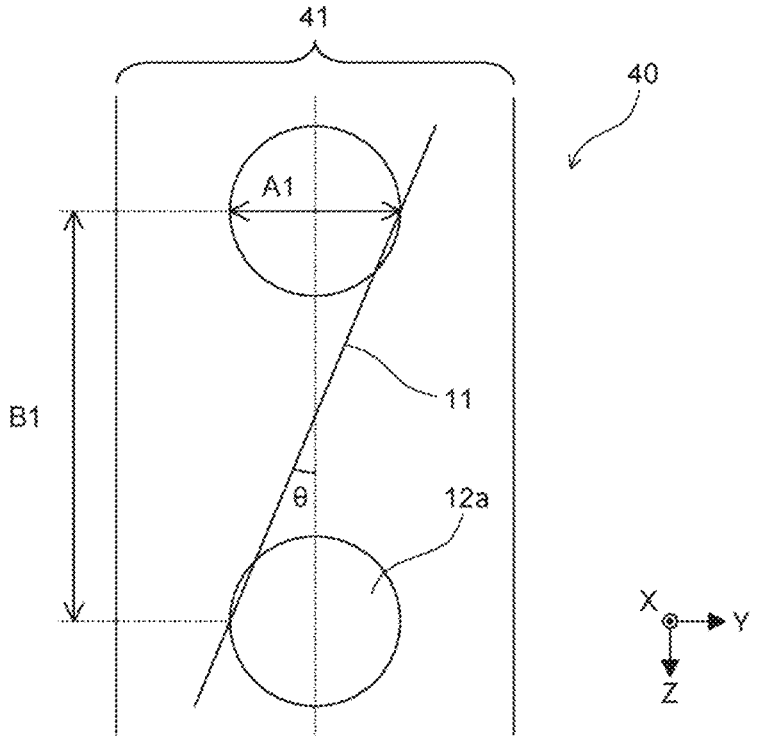


FIG. 14B

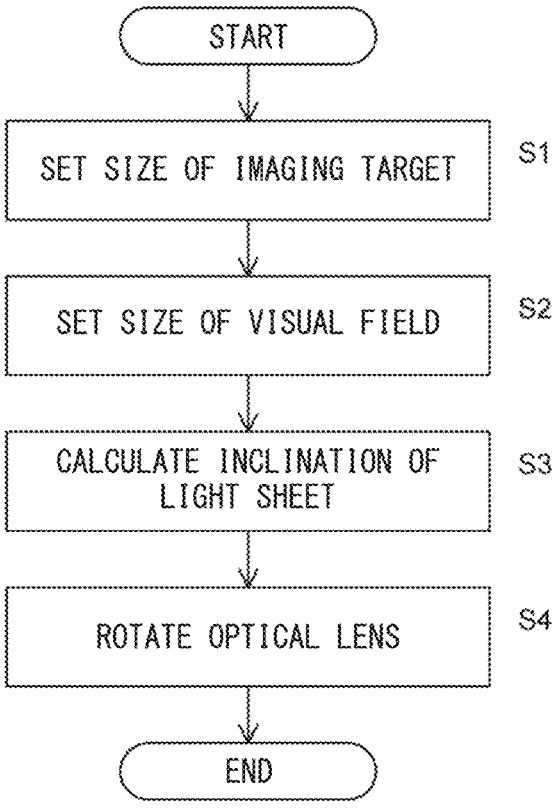


FIG. 15

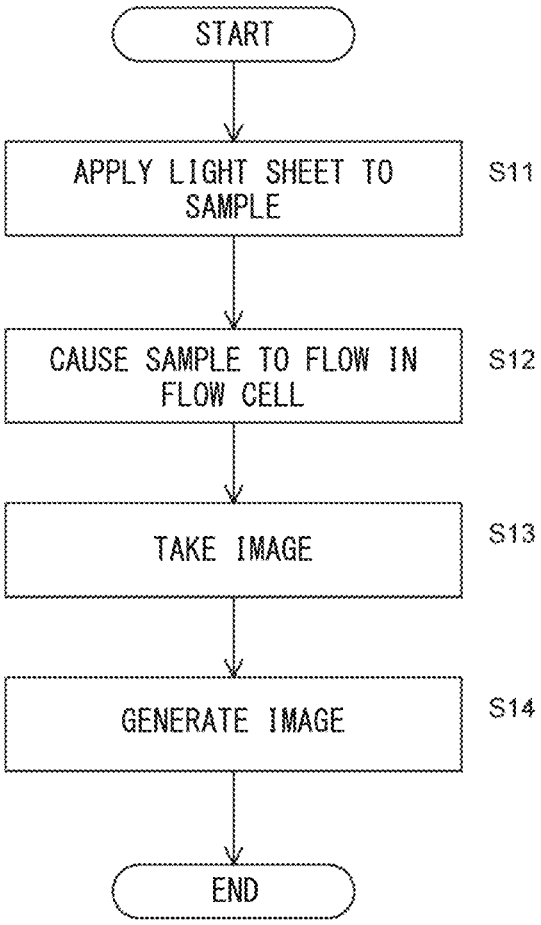

START
APPLY LIGHT SHEET TO SAMPLE
S11
CAUSE SAMPLE TO FLOW IN FLOW CELL
S12
TAKE IMAGE
S13
GENERATE IMAGE
S14
END

FIG. 16

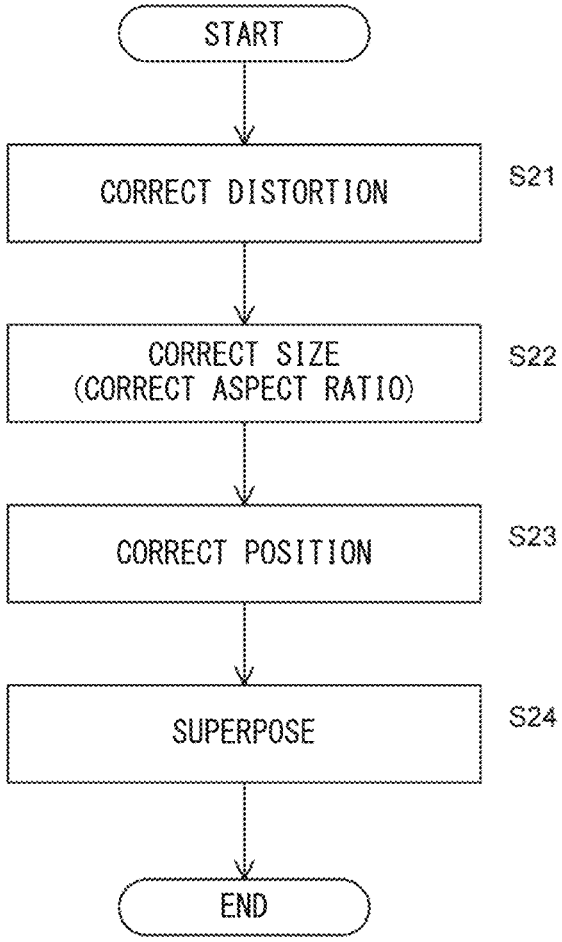

START
CORRECT DISTORTION
S21
CORRECT SIZE
(CORRECT ASPECT RATIO)
S22
CORRECT POSITION
S23
SUPERPOSE
S24
END

FIG. 17

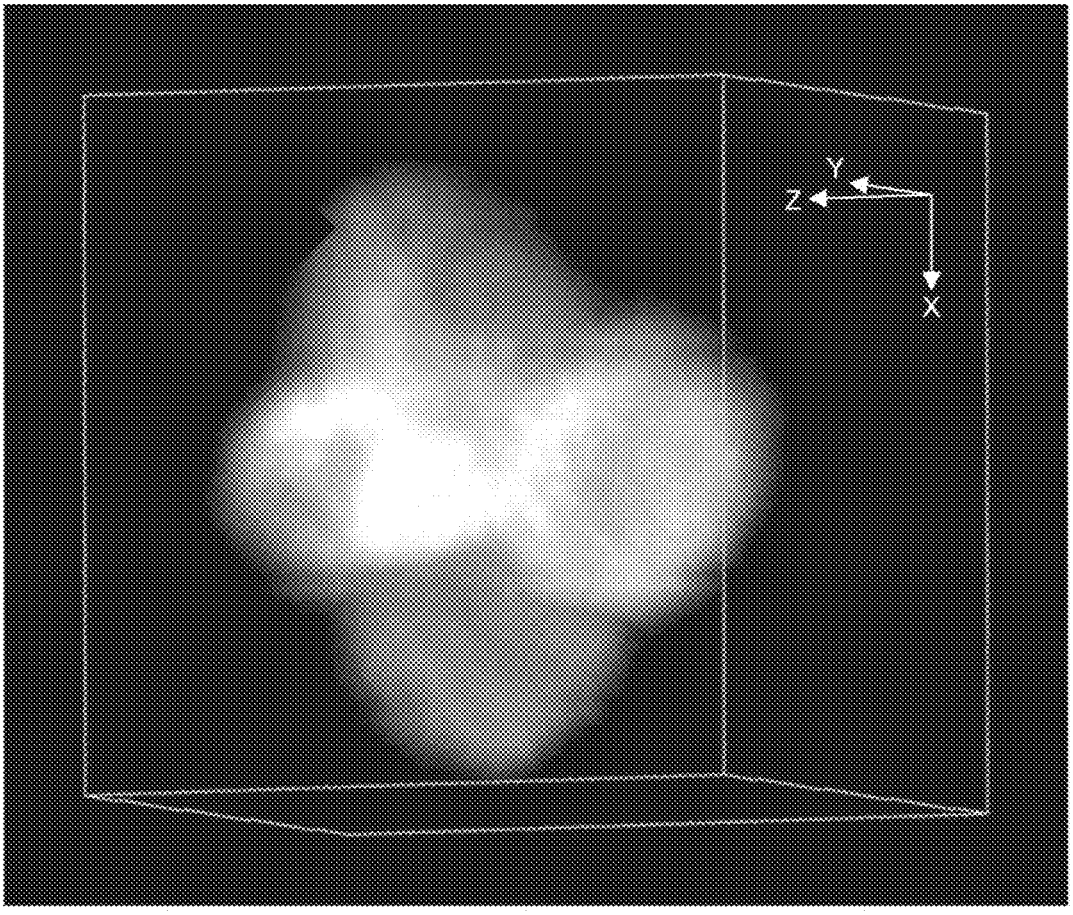


FIG. 18

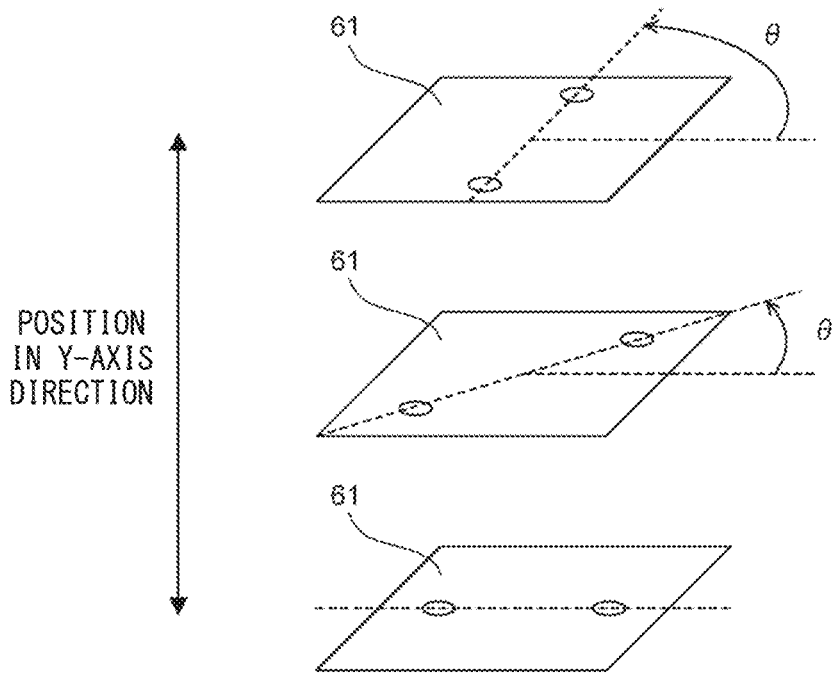


FIG. 19

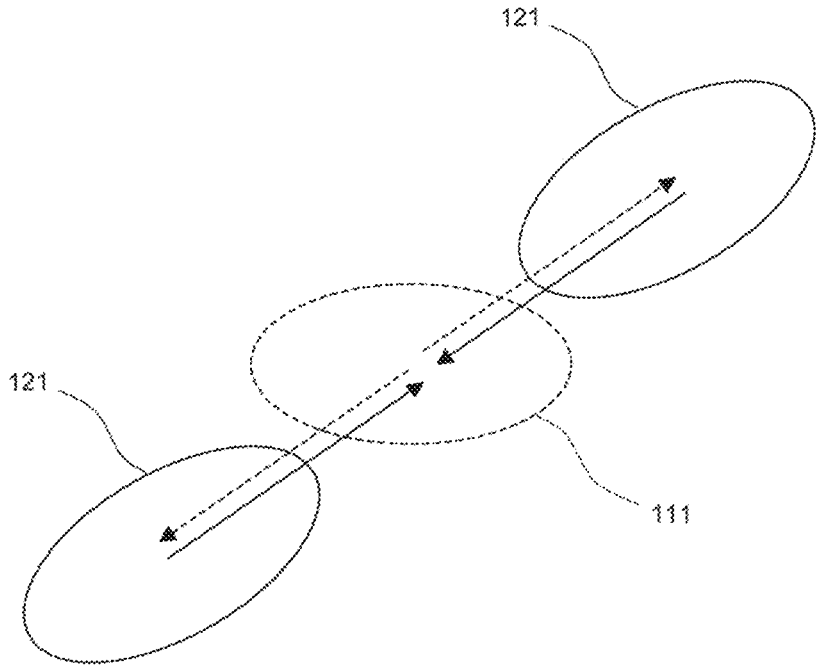


FIG. 20A

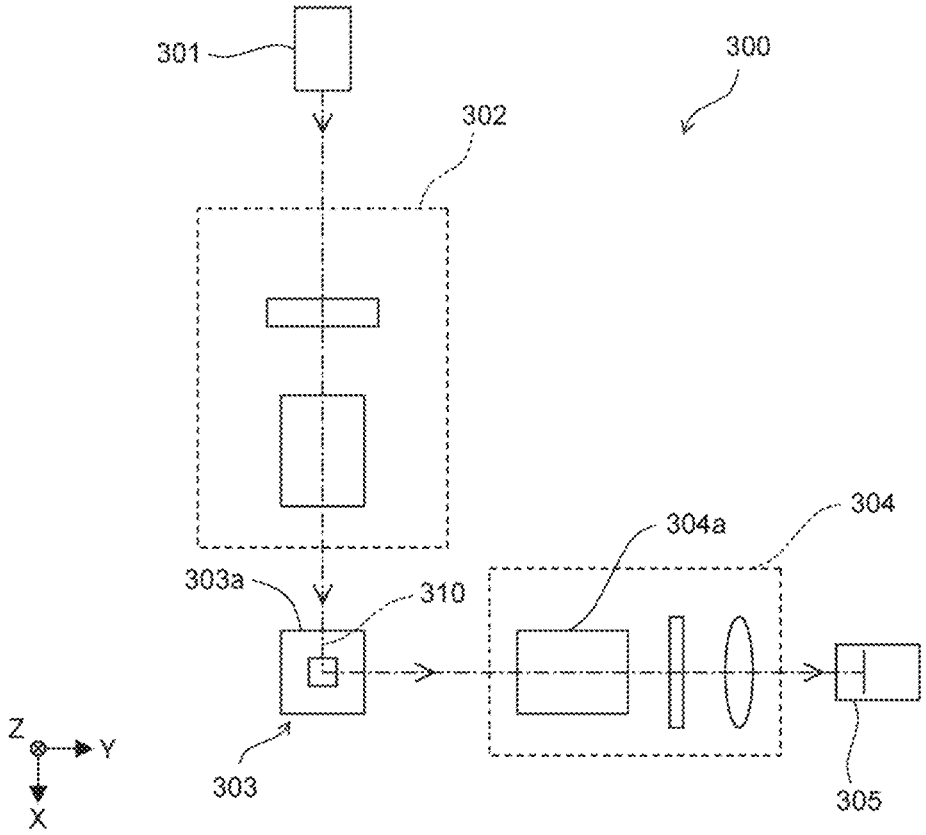
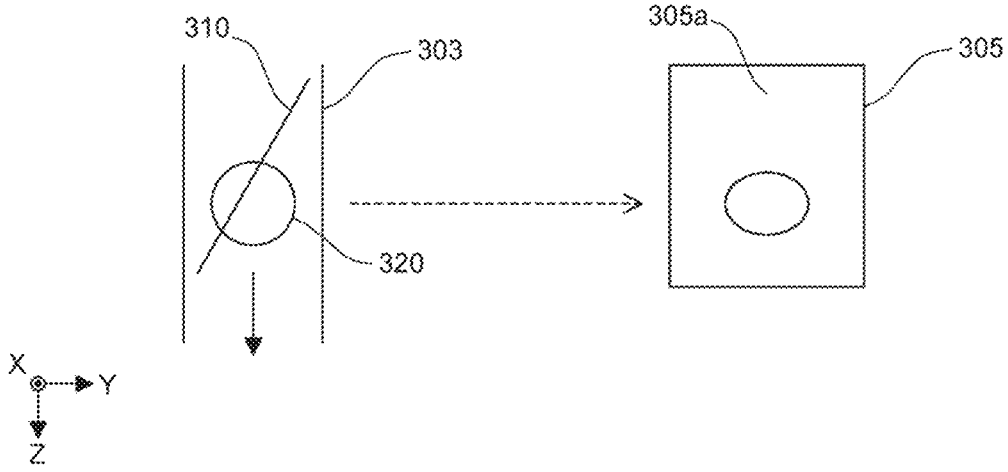


FIG. 20B



CELL IMAGING METHOD, CELL IMAGING APPARATUS, PARTICLE IMAGING METHOD, AND PARTICLE IMAGING APPARATUS

RELATED APPLICATIONS

This application claims priority from prior Japanese Patent Application No. 2017-147660, filed on Jul. 31, 2017, entitled "CELL IMAGING METHOD, CELL IMAGING APPARATUS, PARTICLE IMAGING METHOD, AND PARTICLE IMAGING APPARATUS", the entire content of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a cell imaging method, a cell imaging apparatus, a particle imaging method, and a particle imaging apparatus.

2. Description of the Related Art

Japanese Laid-Open Patent Publication No. 2017-58352 discloses a particle imaging apparatus capable of taking cross-sectional images of particles by inclining a sheet surface of a light sheet with respect to a flow of a sample that flows in a flow cell. That is, as shown in FIGS. **20**A and **20**B, a particle imaging apparatus **300** includes: a light source **301**, an irradiation optical system **302**, a flow cell **303**, a condensing optical system **304**, and an imaging device **305**. Light emitted from the light source **301** is converged by the irradiation optical system **302**, thereby forming a light sheet **310**. A sheet surface of the light sheet **310** is perpendicular to an outer side surface **303***a* of the flow cell **303**, and is inclined at a predetermined angle with respect to a flow direction of a sample that flows in the flow cell **303**. Fluorescence generated from a particle **320** is condensed onto an imaging surface **305***a* of the imaging device **305** by the condensing optical system **304** including an object lens **304***a*.

When cells are imaged, it is desirable to improve throughput so that as many cell images as possible per unit time can be obtained. Demand for improved throughput is particularly high when images of rare cells contained in a sample are taken. In this case, by simultaneously imaging a plurality of cells that simultaneously cross a light sheet, the number of cell images obtainable per unit time can be increased. In this case, however, the taken image of a cell that flows at a position that significantly deviates from a focal position of an object lens becomes unclear due to focus deviation.

When culture stem cells are evaluated, it is useful to image and analyze an aggregate resulting from aggregation of a plurality of cells. In this case, since such an aggregate is larger than a single cell, the aggregate has a relatively large width in the optical axis direction of the object lens. Therefore, a portion of the aggregate may significantly deviate from the focal position of the object lens, and a portion, of the taken image, corresponding to this portion of the aggregate may become unclear.

SUMMARY OF THE INVENTION

The scope of the present invention is defined solely by the appended claims, and is not affected to any degree by the statements within this summary.

A first aspect of the present invention provides a cell imaging method. The cell imaging method according to this aspect includes: forming a light sheet (**11**) with respect to a flow cell (**40**) (S**11**); causing a measurement sample containing a plurality of cells to flow in the flow cell (**40**) (S**12**); receiving lights generated from the plurality of cells passing through the light sheet (**11**), by an imaging device (**60**) via an element (**55**) configured to extend a depth of focus (S**13**), and taking images of the plurality of cells by the imaging device (**60**) (S**14**).

The "depth of focus" is a range of distance on a measurement target side, in which an image taken by the imaging device appears to be in focus, in an optical axis direction of a condensing optical system.

According to the cell imaging method of this aspect, the lights generated from the plurality of cells flowing in the flow cell are imaged via the element configured to extend the depth of focus. Therefore, clear cross-sectional images of the cells can be obtained regardless of the positions of the cells flowing in the flow cell. Accordingly, a high-quality image including the plurality of cells can be generated.

In the cell imaging method of this aspect, the light sheet (**11**) may be formed to be inclined such that the light sheet (**11**) is not perpendicular to a flow direction of the sample. Thus, the lights generated from the plurality of cells can be imaged from the side of the flow cell (**40**).

In the cell imaging method of this aspect, the plurality of cells are caused to simultaneously pass through the light sheet (**11**), and lights generated from the plurality of cells are received by the imaging device (**60**). Thus, the images of the plurality of cells can be simultaneously taken, thereby obtaining an image including the cross-sectional images of the plurality of cells.

In the cell imaging method of this aspect, the element (**55**) configured to extend the depth of focus may be a phase modulation element (**55**) configured to modulate a point spread function.

In this case, the point spread function may be a spiral point spread function. Thereby, the depth of focus can be effectively extended.

The "spiral point spread function" is a point spread function that allows light generated from one bright point to be imaged onto a rotational position on an image surface corresponding to a depth position of the bright point.

The point spread function may be a single-helix point spread function. In this case, since lights generated from portions at the cross section of each cell are not separated, an excellent taken image can be obtained.

The "single-helix point spread function" is a kind of a spiral point spread function, and is a point spread function that allows light generated from one bright point to be imaged on one focal point.

The cell imaging method of this aspect may include subjecting an image including taken images of the plurality of cells to correction of distortion of each taken image, the distortion being caused by the phase modulation element (**55**) (S**21**). By correcting distortion, of the taken image, caused by the effect of the phase modulation element, a high quality cell image can be obtained.

For example, in the correcting of the distortion of the taken image (S**21**), image elements forming the taken image are each shifted to a position at which displacement thereof based on the point spread function is corrected. By individually shifting the image elements, distortion of the entire taken image can be appropriately corrected.

Specifically, in the correcting of the distortion of the taken image (S**21**), each image element is shifted on the basis of

a distance between the light sheet (**11**) and a position, on an imaging surface (**61**), at which the image element is obtained. Thus, distortion of the taken image can be appropriately corrected.

More specifically, in the correcting of the distortion of the taken image (S**21**), each image element is shifted in a direction and by a distance, the direction and the distance being based on the distance between the light sheet (**11**) and the position, on the imaging surface (**61**), at which the image element is obtained. Thus, distortion of the taken image can be appropriately corrected by a simple process.

In this case, each image element may be an image element obtained pixel by pixel. Thus, by setting each image element to be corrected, to an image element obtained from each pixel that is the minimum unit of imaging, distortion of the taken image can be corrected with high accuracy.

Each "image element" is an image portion included in each unit block when a taken image is divided into predetermined unit blocks. Each image element may be an image portion corresponding to one pixel as described above, or may be an image portion included in a unit block composed of a predetermined number of pixels in each of up and down directions.

Further, in the correcting of the distortion of the taken image (S**21**), the image elements obtained from pixel lines (PL**1** to PLn) having the same distance from the light sheet (**11**) may be shifted in a direction and by a distance, the direction and the distance being based on the distance between the pixel lines (PL**1** to PLn) and the light sheet (**11**). Thus, distortion of the taken image can be appropriately corrected by a very simple process.

In the cell imaging method of this aspect, a plurality of images each including a plurality of cells are taken, and an image including three-dimensional images of the plurality of cells is generated on the basis of the plurality of taken images. Thus, high-quality three-dimensional images of the plurality of cells can be obtained.

In the cell imaging method of this aspect, the generating of the image (S**15**) includes correcting a position of the image of each cell at the imaging surface (**61**) (S**23**). In the correcting of the position (S**23**), an amount of shifting of the image of the cell on the imaging surface (**61**) may be calculated on the basis of, at least, an amount of movement of the cell in the flow cell (**40**), and an angle of the light sheet (**11**) with respect to the flow direction of the sample, and the three-dimensional image of the cell may be generated on the basis of the calculated amount of shifting, and a series of the taken images obtained along with movement of the cell. Thus, by generating the three-dimensional images of the plurality of cells in consideration of the amount of shifting of the image at the imaging surface, higher-quality three-dimensional images can be obtained.

Further, the generating of the image (S**21**) includes correcting a size of the image of each cell on the imaging surface (**61**) (S**22**). In the correcting of the size (S**22**), the size of the taken image of the cell is corrected on the basis of an angle of the light sheet (**11**) with respect to the flow direction of the sample, and the three-dimensional image of the cell is generated on the basis of the size-corrected image. Thus, by correcting the size of the taken image, a higher-quality three-dimensional image can be obtained.

A second aspect of the present invention provides a cell imaging apparatus. The cell imaging apparatus according to this aspect includes: a flow cell (**40**) configured to cause a sample containing a plurality of cells to flow therein; a light source (**20**); an irradiation optical system (**30**) configured to form, with respect to the flow cell (**40**), a light sheet (**11**) from light emitted from the light source (**20**); a condensing optical system (**50**) having an element (**55**) configured to extend a depth of focus, the condensing optical system (**50**) being configured to condense lights generated from the plurality of cells flowing in the flow cell (**40**); and an imaging device (**60**) configured to receive lights that have been generated from the plurality of cells and condensed by the condensing optical system (**50**), and take images of the plurality of cells.

According to the cell imaging device of this aspect, the lights generated from the plurality of cells flowing in the flow cell are imaged via the element configured to extend the depth of focus. Therefore, clear cross-sectional images of the cells can be obtained regardless of the positions of the cells flowing in the flow cell. Accordingly, a high-quality image including the plurality of cells can be generated.

In the cell imaging apparatus of this aspect, the element (**55**) configured to extend the depth of focus may be a phase modulation element (**55**) configured to modulate a point spread function.

In this case, the phase modulation element (**55**) may be configured to form a spiral point spread function at an imaging surface (**61**) of the imaging device (**60**). Thus, the depth of focus can be effectively extended.

The spiral point spread function may be a single-helix point spread function. In this case, since lights generated from portions at the cross section of each cell are not separated, an excellent taken image can be obtained.

The cell imaging apparatus of this aspect may include a processing section (**81**) configured to process the images taken by the imaging device (**60**). The processing section (**81**) may be configured to execute a process of correcting distortion of each taken image, the distortion being caused by the phase modulation element (**55**), and generate an image including the plurality of cells on the basis of the distortion-corrected taken images. By correcting distortion, of the taken image, caused by the effect of the phase modulation element, a high quality cell image can be obtained.

For example, the processing section (**81**) may be configured to cause each of image elements forming the taken image to shift to a position at which displacement of the image element based on the point spread function is corrected, thereby correcting the distortion of the taken image. By individually shifting the image elements, distortion of the entire taken image can be appropriately corrected.

Specifically, the processing section (**81**) may be configured to cause each of the image elements forming the taken image to shift, on the basis a distance between the light sheet (**11**) and a position, on an imaging surface (**61**), at which the image element is obtained, thereby correcting the distortion of the taken image. Thus, distortion of the taken image can be appropriately corrected.

More specifically, the processing section (**81**) may be configured to cause the image element to shift in a direction and by a distance, the direction and the distance being based on the distance between the light sheet (**11**) and the position, on the imaging surface (**61**), at which the image element is obtained, thereby correcting the distortion of the taken image. Thus, distortion of the taken image can be appropriately corrected by a simple process.

In this case, each image element may be an image element obtained for each of pixels of the imaging device (**60**). Thus, by setting each image element to be corrected, to an image element obtained from each pixel that is the minimum unit of imaging, distortion of the taken image can be corrected with high accuracy.

The processing section (**81**) may be configured to cause the image elements obtained from pixel lines (PL**1** to PLn) having the same distance from the light sheet (**11**), to shift in a direction and by a distance, the direction and the distance being based on the distance between the pixel lines (PL**1** to PLn) and the light sheet (**11**), thereby correcting the distortion of the taken image. Thus, distortion of the taken image can be appropriately corrected by a very simple process.

Further, the point spread function may be a multi-helix point spread function. In this case, the processing section (**81**) may be configured to cause a plurality of image elements that are paired based on the point spread function to shift to an intermediate position between these image elements, thereby correcting the distortion of the taken image. Thus, by superposing the plurality of image elements that are paired, a bright taken image can be generated.

The "multi-helix point spread function" is a kind of a spiral point spread function, and is a point spread function that allows light generated from one bright point to be imaged on a plurality of focal points.

In the cell imaging apparatus of this aspect, the phase modulation element (**55**) may be a spatial light modulator, a deformable mirror, or a phase plate.

In the cell imaging apparatus of this aspect, the processing section (**81**) may be configured to generate an image including three-dimensional images of the plurality of cells, on the basis of the taken images. Thus, high-quality three-dimensional images of the plurality of cells can be obtained.

In this case, the processing section (**81**) may be configured to calculate an amount of shifting of the image of each cell on an imaging surface (**61**) of the imaging device (**60**), on the basis of, at least, an amount of movement of the cell in the flow cell (**40**), and an angle of the light sheet (**11**) with respect to the flow direction of the sample, and generate the three-dimensional image of the cell on the basis of the calculated amount of shifting, and a series of the taken images obtained along with movement of the cell. Thus, by generating the three-dimensional images of the plurality of cells in consideration of the amount of shifting of the image at the imaging surface, higher-quality three-dimensional images can be obtained.

Further, the processing section (**81**) may be configured to correct a size of each taken image on the basis of an angle of the light sheet (**11**) with respect to the flow direction of the sample, and generate the three-dimensional images of the plurality of cells on the basis of the size-corrected images. Thus, by correcting the size of each taken image, a higher-quality three-dimensional image can be obtained.

The sheet surface (**11***a*) of the light sheet (**11**) may be perpendicular to an outer side surface (**40***a*) of the flow cell (**40**). Thus, light incident on the flow cell is inhibited from being deflected by the flow cell, whereby the shape of the beam that passes through the flow cell and is applied to a cell is less likely to be deformed. Accordingly, the light sheet having an appropriate shape can be applied to the cell, whereby a high-definition image can be taken.

The optical axis of the condensing optical system (**50**) may be perpendicular to the flow direction of the sample. Thus, the imaging device receives light that goes out of the flow cell without being substantially deflected by the flow cell, whereby the beam shape of the light applied to the imaging surface is less likely to be deformed. Therefore, a high-definition image can be imaged by the imaging device.

The optical axis of the irradiation optical system (**30**) and the optical axis of the condensing optical system (**50**) may be perpendicular to each other. Thus, the imaging device can image light emitted from the cross section of each cell, from the front side. In this case, a process of correcting the taken image in the direction perpendicular to the flow of the sample need not be performed.

The irradiation optical system (**30**) may be configured to include: an optical lens (**31**) configured to converge the light emitted from the light source (**20**) such that convergence of the light in a first direction (D**1**) is different from convergence of the light in a second direction (D**2**) that crosses the first direction (D**1**); and a rotation mechanism section (**32**) configured to rotate the optical lens (**31**) about an optical axis of the irradiation optical system (**30**) in the optical lens (**31**). In this configuration, by adjusting the rotation angle of the optical lens, a high-definition image with reduced background noise can be taken while satisfactorily obtaining a series of cross-sectional images of each cell.

A third aspect of the present invention provides a particle imaging method. The particle imaging method according to this aspect includes: forming a light sheet (**11**) with respect to a flow cell (**40**) (S**11**); taking an image of light generated from a particle that flows in the flow cell (**40**), via a phase modulation element (**55**) configured to modulate a point spread function (S**13**); and correcting distortion of the taken image, the distortion being caused by the phase modulation element (**55**) (S**21**).

According to the particle imaging method of this aspect, since the condensing optical system includes the phase modulation element, the depth of focus of the condensing optical system can be extended, whereby a clear cross-sectional image of the particle can be obtained regardless of the position of particle in the optical axis direction of the condensing optical system. Further, since distortion, of the taken image, caused by the effect of the phase modulation element is corrected by a processing section, a high-quality particle image can be obtained. Thus, according to the particle imaging method of this aspect, a higher-quality particle image can be generated regardless of the position of the particle flowing in the flow cell.

A fourth aspect of the present invention provides a particle imaging apparatus. The particle imaging apparatus according to this aspect includes: a flow cell (**40**) configured to cause a sample containing a particle to flow therein; a light source (**20**); an irradiation optical system (**30**) configured to form, with respect to the flow cell (**40**), a light sheet (**11**) from light emitted from the light source (**20**); a condensing optical system (**50**) having a phase modulation element (**55**) configured to extend a depth of focus, the condensing optical system (**50**) being configured to condense light generated from the particle that flows in the flow cell (**40**); an imaging device (**60**) configured to receive the light condensed by the condensing optical system (**50**); and a processing section (**81**) configured to correct distortion of the image taken by the imaging device (**60**).

According to the particle imaging apparatus of this aspect, the same effects achieved by the third aspect can be achieved.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a diagram showing a configuration of a cell imaging apparatus according to Embodiment 1;

FIG. **2**A is a diagram showing a configuration of an optical lens according to Embodiment 1;

FIG. **2**B is a diagram showing a state in which the optical lens according to Embodiment 1 is inclined;

FIG. **2**C is a schematic diagram in which the cross sections of a flow cell and a light sheet in a flow path according to Embodiment 1 are viewed in an X-axis negative direction;

FIG. **2**D is a schematic diagram in which the cross sections of the light sheet and the flow cell according to Embodiment 1 are viewed in a first direction;

FIG. **3**A is a schematic diagram in which the cross-sections of the light sheet and the flow cell according to Embodiment 1 are viewed in a second direction;

FIG. **3**B is a schematic diagram in which the cross sections of the light sheet and the flow cell according to a modification of Embodiment 1 are viewed in the second direction;

FIG. **3**C is a schematic diagram in which the cross sections of the light sheet and the flow cell according to a modification of Embodiment 1 are viewed in the second direction;

FIG. **3**D is a schematic diagram in which the cross sections of the light sheet and the flow cell according to a modification of Embodiment 1 are viewed in the second direction;

FIG. **4**A is a schematic diagram showing the relationship between the sheet surface of the light sheet and the outer side surface of the flow cell according to Embodiment 1;

FIG. **4**B is a schematic diagram showing the relationship between the sheet surface of the light sheet and the outer side surface of the flow cell according to a modification of Embodiment 1;

FIG. **5**A is a diagram showing a phase modulation pattern of a spatial light modulator according to Embodiment 1;

FIG. **5**B is a diagram schematically showing the structure of a phase plate according to Embodiment 1;

FIG. **6** is a diagram showing the structure of an imaging unit in a case where a deformable mirror is used as a phase modulation element, according to Embodiment 1;

FIG. **7**A is a diagram showing an imaging state of an image in an imaging device in a case where a phase modulation element is not provided in a condensing optical system;

FIG. **7**B is a diagram showing an imaging state of an image in the imaging device according to Embodiment 1;

FIG. **8** is a diagram schematically showing distortion of an image caused by an effect of the phase modulation element, according to Embodiment 1;

FIG. **9** is a diagram schematically showing shifting of irradiation positions caused by the effect of the phase modulation element, according to Embodiment 1;

FIG. **10**A is a diagram showing the effect of the phase modulation element according to Embodiment 1;

FIG. **10**B is a diagram showing the effect of the phase modulation element according to Embodiment 1;

FIG. **10**C is a diagram showing the effect of the phase modulation element according to Embodiment 1;

FIG. **11**A is a diagram explaining a process of correcting image elements in each of pixel lines of the imaging device, according to Embodiment 1;

FIG. **11**B is a table showing the contents of correction vectors according to Embodiment 1;

FIG. **12**A is a diagram explaining aspect-ratio correction and position adjustment when images obtained by the imaging device according to Embodiment 1 are superposed on one another;

FIG. **12**B is a diagram explaining aspect-ratio correction and position adjustment when images obtained by the imaging device according to Embodiment 1 are superposed on one another;

FIG. **13**A is a diagram explaining the relationship between inclination of the light sheet and imaging accuracy, according to Embodiment 1;

FIG. **13**B is a diagram explaining the relationship between inclination of the light sheet and imaging accuracy, according to Embodiment 1;

FIG. **14**A is a diagram explaining the conditions for an angle that allows obtainment of all cross sections while reducing noise component, according to Embodiment 1;

FIG. **14**B is a flowchart showing process steps for applying an optimum angle to the cell imaging apparatus according to Embodiment 1;

FIG. **15** is a flowchart showing process steps for generating a three-dimensional image according to Embodiment 1;

FIG. **16** is a flowchart showing the content of an image generation process according to Embodiment 1;

FIG. **17** is a diagram showing an example of a three-dimensional image in which a plurality of cells are aggregated, according to Embodiment 1;

FIG. **18** is a diagram explaining an effect of a phase modulation element according to Embodiment 2;

FIG. **19** is a diagram explaining a cross-sectional image correcting method according to Embodiment 2;

FIG. **20**A is a diagram explaining the configuration of a related art; and

FIG. **20**B is a diagram schematically showing portions corresponding to a flow cell and an imaging device in the configuration of the related art.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Embodiment 1

Embodiment 1 is a cell imaging apparatus configured to image fluorescences generated from a cell that is irradiated with light, thereby obtaining a plurality of images, and configured to generate a three-dimensional image of the cell on the basis of the obtained plurality of images. Examples of the imaging target cell include: a circulating tumor cell (CTC); a circulating endothelial cell (CEC); an endothelial progenitor cell (EPC); an mesenchymal stem cell (MSC); a hematopoietic stem cell (HSC); and an antigen-specific T-cell.

In Embodiment 1, a fluorescence image of a nucleus in the imaging target cell is obtained.

As shown in FIG. **1**, a cell imaging apparatus **10** includes an imaging unit **10***a* and an information processing unit **10***b*. The imaging unit **10***a* includes a light source **20**, an irradiation optical system **30**, a flow cell **40**, a condensing optical system **50**, an imaging device **60**, and a rotation controller **70**. In FIG. **1**, XYZ axes are shown for the purpose of explaining arrangement of the respective components of the imaging unit **10***a*. The XYZ axes are orthogonal to each other. XYZ axes shown in the following drawings correspond to the XYZ axes shown in FIG. **1**.

The irradiation optical system **30** includes an optical lens **31**, a rotation mechanism section **32**, and an object lens **33**. The condensing optical system **50** includes an object lens **51**, an optical filter **52**, condenser lenses **53**, **54**, and **56**, and a phase modulation element **55**. In this embodiment, a transmission type phase modulation element **55** is assumed.

The light source **20** emits light in the X-axis positive direction to irradiate a sample flowing in the flow cell **40** with the light. The light source **20** is, for example, a semiconductor laser light source. The wavelength of the

light emitted from the light source **20** is set to a wavelength of light for exciting fluorescence from a fluorescent dye that stains each cell. The optical lens **31** converges the light emitted from the light source **20** as described later. The rotation mechanism section **32** rotatably supports the optical lens **31**. The rotation mechanism section **32** causes the optical lens **31** to rotate about the center axis of the light emitted from the light source **20**, that is, about the optical axis of the irradiation optical system **30** in the optical lens **31**.

As shown in FIG. **2**A, the optical lens **31** is a cylindrical lens. The X-axis positive side of the optical lens **31** is a flat surface, while the X-axis negative side of the optical lens **31** is a curved surface. The optical lens **31** is supported by the rotation mechanism section **32** such that the X-axis positive side surface thereof is perpendicular to the X-axis, and the center axis of the light incident on the optical lens **31** crosses a generatrix **31***a* of the optical lens **31**. The optical lens **31** is preferably arranged such that the flat surface thereof is positioned on the X-axis positive side while the curved surface thereof is positioned on the X-axis negative side as described above. However, the optical lens **31** may be arranged such that the curved surface is positioned on the X-axis positive side while the flat surface is positioned on the X-axis negative side.

The optical lens **31** converges the light emitted from the light source **20** such that convergence of the light in the first direction D**1** is different from convergence of the light in a second direction D**2** that crosses the first direction D**1**. Specifically, the first direction D**1** is a direction perpendicular to the generatrix **31***a* and the X-axis, and the second direction D**2** is a direction parallel to the generatrix **31***a*. The optical lens **31** does not converge the light emitted from the light source **20** in the second direction D**2**, but converges the light only in the first direction D**1**. The light converged in the first direction D**1** by the optical lens **31** is condensed on and around a pupil of the object lens **33**.

As shown in FIG. **2**B, the optical lens **31** is rotated about the X-axis by the rotation mechanism section **32**, and is located at a rotational position at which the angle of the generatrix **31***a* with respect to the Y-axis is a predetermined angle θ. Thus, the optical lens **31** converges the light emitted from the light source **20** only in the first direction D**1** inclined with respect to the Z-axis as shown in FIG. **2**B.

Referring back to FIG. **1**, the object lens **33** causes the light transmitted through the optical lens **31** to be condensed onto a flow path **41** of the flow cell **40**. Specifically, the object lens **33** converges the light transmitted through the optical lens **31** such that the convergence position of the light in the second direction D**2** shown in FIG. **2**B is positioned in the flow path **41** of the flow cell **40**. In addition, the object lens **33** collimates the light transmitted through the optical lens **31**, in the first direction D**1** shown in FIG. **2**B. Thus, the light transmitted through the object lens **33** becomes a flat beam in the flow path **41** of the flow cell **40**.

The object lens **33** may be omitted. In this case, the optical lens **31**, being in the state shown in FIG. **2**B, is rotated by 90° about the X-axis. Then, the light emitted from the light source **20** is converged by the optical lens **31** only in one direction, whereby a flat beam is formed in the flow path **41** of the flow cell **40**.

As described above, the irradiation optical system **30**, by means of the optical lens **31** and the object lens **33**, causes the light emitted from the light source **20** to be linearly condensed onto a cross section parallel to the YZ plane at the position of the flow path **41** of the flow cell **40**. That is, the irradiation optical system **30** forms a light sheet **11** with respect to the flow cell **40**, from the light emitted from the light source **20**.

The optical lens **31** may be a lens that causes convergence of the light in the first direction D**1** to be different from convergence of the light in the second direction D**2**. The optical lens **31** may be a phase plate or a holography element. The irradiation optical system **30** may form the light sheet **11** by forming a Bessel beam by using a conical lens or the like, and scanning the formed Bessel beam at a high speed in one direction by using a scanning mirror or the like. In this case, the scanning direction of the scanning mirror or the like is, in the YZ plane, a direction other than the Y-axis direction and the Z-axis direction.

The flow cell **40** has a shape extending in the Z-axis direction, and has a cross section of a square outer shape as viewed in the Z-axis direction. The flow cell **40** may have a cross section of an outer shape that is a rectangle other than a square as viewed in the Z-axis direction. Outer side surfaces **40***a*, **40***b*, **40***c*, and **40***d* of the flow cell **40** are flat surfaces. In particular, the outer side surface **40***a* on which the light from the irradiation optical system **30** is incident, and the outer side surface **40***b* through which fluorescence condensed by the condensing optical system **50** described later passes, are desired to be flat surfaces. In Embodiment 1, the outer side surface **40***c* of the flow cell **40** on the X-axis positive side and the outer side surface **40***a* of the flow cell **40** on the X-axis negative side, are parallel to the YZ plane, while the outer side surface **40***b* of the flow cell **40** on the Y-axis positive side and the outer side surface **40***d* of the flow cell **40** on the Y-axis negative side, are parallel to the XZ plane.

The flow path **41** extending in the Z-axis direction is formed in the flow cell **40**. The flow cell **40** causes a sample containing cells to flow in the flow path **41**. The sample that flows in the flow path **41** has been prepared in advance on the basis of cells collected from a subject. In Embodiment 1, when the sample is prepared, nuclei in the cells are fluorescently stained. The nuclei are stained by fluorescent dyes that can specifically stain the nuclei. The dyes that stain the nuclei cause excitation of fluorescences having different wavelengths when being irradiated with the light emitted from the light source **20**. When cells that intrinsically generate fluorescences are to be imaged, these cells are not necessarily fluorescently stained.

As shown in FIG. **2**C, when the flow path **41** is viewed in the X-axis negative direction, the longitudinal direction of the light sheet **11** is not perpendicular to the sample flow direction but is inclined at a predetermined angle. That is, the light sheet **11** has a shape extending in the first direction D**1** and having a narrow width in the second direction D**2**. The light sheet **11** is schematically shown by a long chain line. Each cell **12** contained in the sample flows in the Z-axis positive direction in the flow path **41** of the flow cell **40**. At this time, a nucleus **12***a* in the cell **12** also flows in the Z-axis positive direction in the flow path **41**. When the cell **12** crosses the light sheet **11**, fluorescence is generated from a fluorescently stained portion of the cell **12**.

When a cross section C**1**-C**2** in FIG. **2**C is viewed in the first direction D**1**, the cross section is as shown in FIG. **2**D. As shown in FIG. **2**D, the light incident on the flow cell **40** from the irradiation optical system **30** is, in the flow cell **40**, parallel light whose width in the first direction D**1** is not narrowed, but the width thereof in the second direction D**2** is narrowed and thinned. The light sheet **11** is a region, of the light emitted from the light source **20**, whose width in the second direction D**2** is sufficiently narrow relative to the cell.

A sheet surface **11***a* of the light sheet **11** is a plane, in the light sheet **11**, defined by the center axis of the light sheet **11** and the first direction D**1**. In FIGS. **2**C and **2**D, the sheet surface **11***a* is schematically shown by a dotted line.

Referring back to FIG. **2**C, the sheet surface **11***a* of the light sheet **11** is inclined by an angle θ with respect to the Z-axis, in response to inclination of the optical lens **31**. The inclination of the sheet surface **11***a* with respect to the Z-axis is set so as not to be substantially perpendicular to the Z-axis. Thus, a cross-sectional image of the cell **12** can be easily obtained from the periphery of the flow cell **40**. In addition, the sheet surface **11***a* is set so as not to be parallel to the Z-axis. Thus, a plurality of different cross-sectional images can be obtained for the fluorescently stained portion of the cell **12**. Thus, the sheet surface **11***a* is not substantially perpendicular to the Z-axis direction which is the sample flow direction, but is inclined at the predetermined angle. Therefore, fluorescence generated from the fluorescent dye can be easily obtained from the periphery of the flow cell **40**, and a plurality of different cell cross sections can be obtained. The angle θ can be set by rotating the optical lens **31** about the X-axis.

The optical axis of the irradiation optical system **30** is perpendicular to the Z-axis direction which is the sample flow direction. In other words, the optical axis of the object lens **33** is perpendicular to the Z-axis, and the central axis of the light that goes out of the irradiation optical system **30** and is incident on the flow cell **40** is perpendicular to the Z-axis. When a cross section C**3**-C**4** shown in FIG. **2**C is viewed in the second direction D**2**, the cross section is as shown in FIG. **3**A. As shown in FIG. **3**A, the sheet surface **11***a* of the light sheet **11** is perpendicular to the outer side surface **40***a* of the flow cell **40** on which the light emitted from the light source **20** is incident. Thus, the light incident on the flow cell **40** is inhibited from being deflected by the flow cell **40**, whereby the shape of the beam that passes through the flow cell **40** and is applied to the nucleus **12***a* is less likely to be deformed. Accordingly, the light sheet **11** having an appropriate shape can be applied to the cell, thereby enabling the imaging device **60** described later to take a high-definition image.

When a three-dimensional image of only a portion of the nucleus **12***a* is required, the width of the light sheet **11** in the first direction D**1** may be set such that the light sheet **11** covers only the imaging-target portion of the nucleus **12***a*, as shown in FIG. **3**B. In this case, cross-sectional images of the portion of the nucleus **12***a* are obtained, and a three-dimensional image is generated on the basis of the obtained cross-sectional images of the portion of the nucleus **12***a*.

The optical axis of the irradiation optical system **30** may deviate from the perpendicular state with respect to the sample flow direction. In this case, when a cross section C**3**-C**4** shown in FIG. **2**C is viewed in the second direction D**2**, the cross section is as shown in FIG. **3**C. In FIG. **3**C, the optical axis of the irradiation optical system **30** is not perpendicular to the sample flow direction, but, as in FIG. **3**A, the sheet surface **11***a* is perpendicular to the outer side surface **40***a* of the flow cell **40** on which the light emitted from the light source **20** is incident. In this case, due to the outer side surface **40***a*, the light sheet **11** is deflected in the first direction D**1**, but is not deflected in the second direction D**2**. Accordingly, the thickness of the light sheet **11** in the second direction D**2** is less likely to be affected by the outer side surface **40***a* of the flow cell **40**. Therefore, as in the case of FIG. **3**A, the shape of the beam applied to the nucleus **12***a* is less likely to be deformed, thereby enabling the imaging device **60** to take a high-definition image.

When a three-dimensional image of only a portion of the nucleus **12***a* is required, the width of the light sheet **11** in the first direction D**1** may be set such that the light sheet **11** covers only the imaging target portion of the nucleus **12***a*, as shown in FIG. **3**D.

The sheet surface **11***a* may slightly deviate from the perpendicular state to the outer side surface **40***a*. As long as the sheet surface **11***a* is substantially perpendicular to the outer side surface **40***a*, the shape of the beam applied to the cell **12** is inhibited from being deformed, thereby enabling the imaging device **60** to take a high-definition image.

The state in which the sheet surface **11***a* of the light sheet **11** is perpendicular to the outer side surface **40***a* is described in detail with reference to FIGS. **4**A and **4**B.

In Embodiment 1, the optical axis of the irradiation optical system **30** is parallel to the X-axis, and the outer side surface **40***a* of the flow cell **40** is parallel to the YZ plane. Thus, as shown in FIG. **4**A, a central axis **90** of light incident on the outer side surface **40***a* is perpendicular to the outer side surface **40***a*, and the sheet surface **11***a* of the light sheet **11** is perpendicular to the outer side surface **40***a*. In this case, as described above with reference to FIG. **3**A, the thickness of the light sheet **11** in the second direction D**2** is less likely to be affected by the outer side surface **40***a*, thereby enabling the imaging device **60** to take a high-definition image.

However, the central axis **90** of the light incident on the outer side surface **40***a* is not necessarily perpendicular to the outer side surface **40***a*. Specifically, the central axis **90** shown in FIG. **4**A may be inclined in the first direction D**1**. Thus, as shown in FIG. **4**B, the central axis **90** of the light incident on the outer side surface **40***a* is not perpendicular to the outer side surface **40***a*, but the sheet surface **11***a* of the light sheet **11** is perpendicular to the outer side surface **40***a*. Also in this case, as described above with reference to FIG. **3**C, the thickness of the light sheet **11** in the second direction D**2** is less likely to be affected by the outer side surface **40***a*, thereby enabling the imaging device **60** to take a high-definition image.

Referring back to FIG. **1**, the condensing optical system **50** condenses the fluorescence generated from the cell **12**, at the Y-axis positive side of the flow cell **40**. The condensing optical system **50** may condense the fluorescence generated from the cell **12**, at the Y-axis negative side of the flow cell **40**. The object lens **51** condenses the fluorescence generated from the cell **12**. The optical filter **52** blocks unnecessary light such as side scattered light generated from the cell **12**, and causes only the fluorescence to be imaged to pass therethrough. If such unnecessary light is not concerned, the optical filter **52** may be omitted. The condenser lens **53** condenses the fluorescence transmitted through the optical filter **52**. Depending on the specification of the object lens **51**, the condenser lens **53** may be omitted.

The condenser lenses **54** and **56** form a Fourier plane in the condensing optical system **50**. A Fourier plane may be formed by further disposing an even number of lenses between the condenser lens **56** and the phase modulation element **55**.

The phase modulation element **55** is disposed on the Fourier plane in the condensing optical system **50**, and modulates the phase of light to realize an extended depth of focus (EDoF). The phase modulation element **55** forms a point spread function (PSF) for obtaining the extended depth of focus. That is, the phase modulation element **55** has an effect of modulating the PSF to realize the extended depth of focus. The phase modulation element **55** is a phase modulation element that forms a spiral point spread function.

The phase modulation element **55** forms a PSF that allows light generated from a single point to be imaged onto a single focal point. Such a PSF is called SH-PSF (Single-Helix Point Spread Function). The spiral point spread function formed by the phase modulation element **55** is a single-helix point spread function. The configuration of the phase modulation element **55** will be described later with reference to FIGS. **5**A and **5**B.

The imaging device **60** receives, at an imaging surface **61**, the fluorescence condensed by the condensing optical system **50**. The imaging device **60** takes a two-dimensional image of the fluorescence, and outputs the taken two-dimensional image. The taken two-dimensional image is a cross-sectional image of the cell **12**. The imaging device **60** is implemented by, for example, a color CCD. In Embodiment 1, since fluorescence having a predetermined wavelength is generated from the nucleus **12***a*, the imaging device **60** is configured to be able to identify at least light having this wavelength. In a case where the cell is stained by a plurality of kinds of fluorescent dyes, the imaging device **60** is configured to be able to identify lights having different wavelengths. If satisfactory sensitivity cannot be obtained by a color CCD, adjustment may be performed, such as sufficiently reducing the speed of the sample that flows in the flow cell **40**.

The fluorescences having the respective wavelength bands may be separated for each wavelength band in the condensing optical system **50**, and each of the separated fluorescences may be received by an imaging device or a color CCD capable of identifying only light of one wavelength band. In this case, images obtained by a plurality of imaging devices at the same timing are superposed on one another, thereby generating a single cross-sectional image. In a case where only fluorescence of one wavelength band is generated from the cell **12**, the imaging device **60** may be configured to be able to identify only light of one wavelength band.

The optical axis of the condensing optical system **50** is perpendicular to the Z-axis direction which is the sample flow direction. In other words, the optical axis of the object lens **51** is perpendicular to the Z-axis. Thus, the imaging device **60** receives a portion, of the fluorescence generated from the cell **12**, which goes out of the flow cell **40** without being substantially deflected by the flow cell **40**, whereby the beam shape of the fluorescence applied to the imaging surface **61** is less likely to be deformed. Accordingly, a high-definition image can be taken by the imaging device **60**.

The optical axis of the condensing optical system **50** may slightly deviate from the perpendicular state to the sample flow direction. As long as the optical axis of the condensing optical system **50** is substantially perpendicular to the sample flow direction, the beam shape of the fluorescence applied to the imaging surface **61** is inhibited from being deformed, whereby a high-definition image can be taken by the imaging device **60**.

The optical axis of the irradiation optical system **30** and the optical axis of the condensing optical system **50** are perpendicular to each other. Thus, the imaging device **60** can image the fluorescence generated from the cross section of the cell **12**, from the front side. That is, the imaging device **60** images the fluorescence not at a position that deviates in the X-axis direction with respect to the cross section of the cell **12** but in the YZ plane including the cross section of the cell **12**. Thus, a process of correcting the taken image in the X-axis direction need not be performed.

The optical axis of the irradiation optical system **30** and the optical axis of the condensing optical system **50** may slightly deviate from the state of being perpendicular to each other. As long as the optical axis of the irradiation optical system **30** and the optical axis of the condensing optical system **50** are substantially perpendicular to each other, the imaging device **60** can image, from substantially the front side, the fluorescence generated from the cross section of the cell **12**, which substantially eliminates the need for performing the process of correcting the taken image in the X-axis direction.

The rotation controller **70** is connected to the rotation mechanism section **32**, and controls rotation of the rotation mechanism section **32**. Control by the rotation controller **70** will be described later with reference to FIGS. **14**A and **14**B.

The information processing unit **10***b* includes a processing section **81**, a storage section **82**, a display section **83**, an input section **84**, and an interface **85**. The processing section **81** is implemented by, for example, a CPU. The storage section **82** is implemented by, for example, a ROM, a RAM, or a hard disk. The processing section **81** controls the respective components in the information processing unit **10***b* via the interface **85**, and controls the imaging device **60** and the rotation controller **70**.

The processing section **81** generates a three-dimensional image on the basis of the images obtained by the imaging device **60**. Specifically, the imaging device **60** generates a three-dimensional image by superposing a plurality of cross-sectional images obtained from one cell. The display section **83** is a display for displaying, for example, the processing result of the processing section **81**. The input section **84** is a keyboard and a mouse for receiving an input of instruction by an operator.

Next, the configuration of the phase modulation element **55** is described.

The phase modulation element **55** performs phase modulation on fluorescence that transmits therethrough. As for the phase modulation element **55**, a transmission type spatial light modulator using a liquid crystal panel can be used, for example. The spatial light modulator is capable of performing phase modulation at 256 gray levels for each pixel. A phase modulation pattern for forming a single-helix PSF is set as shown in FIG. **5**A, for example. The phase modulation pattern is a pattern distribution of gray levels set for all pixels.

In FIG. **5**A, pixels having the gray level of 0 are shown in black, and pixels having the gray level of 255 are shown in white. Each pixel having the gray level of 0 does not modulate the phase of the incident fluorescence. The phase of the fluorescence incident on each pixel having the gray level of 255 is shifted by 2π with respect to the phase of the fluorescence incident on the pixel having the gray level of 0.

As shown in FIG. **5**B, a phase plate can also be used as the phase modulation element **55**. The phase plate is formed of a transparent material such as acrylic resin. The material forming the phase plate is not necessarily transparent. Any material can be used as long as it can transmit light. When the thickness T**11** of the phase plate varies, the phase of the fluorescence transmitting therethrough varies. The thickness T**11** of each portion of the phase plate is adjusted such that phase modulation similar to the phase modulation pattern shown in FIG. **5**A is generated.

A deformable mirror can also be used as the phase modulation element **55**. When a deformable mirror is used as the phase modulation element **55**, the configuration of the imaging unit **10***a* is changed as shown in FIG. **6**. Also in this case, the phase modulation element **55** is disposed on the

Fourier plane of the condensing optical system **50**. The fluorescence condensed by the object lens **51** is reflected by the phase modulation element **55**, thereby being subjected to phase modulation. Thus, a single-helix PSF is formed at the imaging surface **61** of the imaging device **60**. In the configuration of FIG. **6**, a reflection type spatial light modulator may be used as the phase modulation element **55**. In this case, for example, a polarizer is disposed between the object lens **51** and the optical filter **52**.

As described above, the depth of focus of the condensing optical system **50** can be extended by providing the phase modulation element **55** in the condensing optical system **50**.

FIG. **7**A schematically shows an imaging state in a case where the phase modulation element **55** is not provided in the condensing optical system **50**. In FIG. **7**A, three cells **12** flow in the flow path **41** of the flow cell **40**. The flow path **41** of the flow cell **40** has a width enough to allow a plurality of cells to simultaneously flow at positions in the width direction. The light sheet **11** has a width that covers at least the entirety of the flow path **41**. The imaging device **60** has a width enough to receive at least light from the region of the light sheet **11** included in the flow path **41**. The three cells **12** simultaneously cross the light sheet **11**. The cross sections, of the nuclei **12***a* of the three cells **12**, cut by the light sheet **11** are simultaneously imaged by the imaging device **60**.

The positions at which the three cells **12** flow are shifted from each other in the Y-axis direction. The upper and lower cells **12** are shifted by distances AS**1** and AS**2**, respectively, with respect to the center cell **12** in the Y-axis direction. In a case where the focus position of the condensing optical system **50** is set at the center of the flow path **41** in the Y-axis direction, the cross-sectional image of the nucleus **12***a* of the center cell **12** is satisfactorily taken. However, regarding the upper and lower cells **12**, since these cells are shifted from the focus position of the condensing optical system **50**, the cross-sectional images of the nuclei **12***a* thereof cannot be satisfactorily taken. On the right side in FIG. **7**A, the cross-sectional images of the nuclei **12***a* of the three cells **12** are schematically shown.

On the other hand, in the case where the phase modulation element **55** that forms the spiral PSF is provided in the condensing optical system **50**, the depth of focus of the condensing optical system **50** is extended. Therefore, as shown in FIG. **7**B, even when the positions of the upper and lower cells **12** are shifted from the center of the flow path **41** in the Y-axis direction, that is, in the optical axis direction of the condensing optical system **50**, it is possible to satisfactorily obtain the cross-sectional images of the nuclei **12***a* of these cells **12**. Thus, regarding the plurality of cells **12** simultaneously crossing the light sheet **11**, high-quality cross-sectional images of the nuclei **12***a* thereof can be obtained.

Also in one cell **12**, regarding the distance between the cross section of the nucleus **12***a* cut by the light sheet **11** and the imaging surface of the imaging device **60**, the distance on the Z-axis positive side of the cross section is short and the distance on the Z-axis negative side of the cross section is long. Therefore, when the phase modulation element **55** is not provided in the condensing optical system **50**, partial focus deviation may occur also within the cross-sectional image of one nucleus **12***a*. In contrast, when the phase modulation element **55** is provided in the condensing optical system **50**, since the depth of focus of the condensing optical system **50** is extended as described above, partial focus deviation does not occur within the cross-sectional image of one nucleus **12***a*. Therefore, an excellent cross-sectional image of the nucleus **12***a* can be obtained.

However, when the phase modulation element **55** is provided in the condensing optical system **50**, distortion occurs in the cross-sectional image of the nucleus **12***a* due to the phase modulation effect of the phase modulation element **55**.

FIG. **8** schematically shows this distortion. With reference to FIG. **8**, for convenience, distortion of the cross-sectional image of one nucleus **12***a* is described for a case where the one nucleus **12***a* crosses the light sheet **11** while flowing along the flow path **41**. In this case, with movement of the nucleus **12***a*, the position of the cross section of the nucleus **12***a* cut by the light sheet **11** changes in the Y-axis direction. That is, the position of the cross section of the nucleus **12***a* is more displaced in the Y-axis negative direction when the nucleus **12***a* is located at a position **102** than when the nucleus **12***a* is located at a position **101**. Further, the position of the cross section of the nucleus **12***a* is more displaced in the Y-axis negative direction when the nucleus **12***a* is located at a position **103** than when the nucleus **12***a* is located at the position **102**. Strictly speaking, regarding the cross section of the nucleus **12***a* cut by the light sheet **11**, the distance between each portion in the cross section and the imaging surface of the imaging device **60** changes depending on the position of the cross section in the Z-axis direction.

When the phase modulation element **55** is not provided in the condensing optical system **50**, fluorescences generated from these cross sections are applied to irradiation areas **111**, **112**, and **113** indicated by broken lines, respectively, on the imaging surface **61** of the imaging device **60**. However, when the phase modulation element **55** is provided in the condensing optical system **50**, the fluorescences generated from the respective cross sections are applied to irradiation areas **121**, **122**, and **123** indicated by solid lines, respectively, due to the phase modulating effect of the phase modulation element **55**. The irradiation areas **121**, **122**, and **123** are displaced and deformed in the direction of the arrow shown in FIG. **8** with respect to the irradiation areas **111**, **112**, and **113**. This distortion is caused by that each portion in the cross section of the nucleus **12***a* is shifted in the direction according to the distance between the portion and the imaging surface **61**, due to the effect of the single-helix PSF. That is, this distortion is based on the effect of the single-helix PSF formed by the phase modulation element **55**.

As shown in FIG. **9**, when the phase modulation element **55** is not provided in the condensing optical system **50**, fluorescences generated from portions of the cross section of the nucleus **12***a* are applied to, for example, positions P**11**, P**12**, and P**13** in the irradiation area **111**. On the other hand, when the phase modulation element **55** is provided in the condensing optical system **50**, fluorescences generated from the portions of the cross section of the nucleus **12***a* are applied to positions P**21**, P**22**, and P**23** in the irradiation area **121**. Thus, when the phase modulation element **55** is provided in the condensing optical system **50**, the irradiation positions of the fluorescences generated from the respective portions of the cross section of the nucleus **12***a* are shifted with respect to those in the case where the phase modulation element **55** is not provided in the condensing optical system **50**.

As shown in FIGS. **10**A to **10**C, when the phase modulation element **55** forms the single-helix PSF, the positional relationship between the fluorescence irradiation positions F**11**, F**12**, and F**13** on the imaging surface **61** in the case where the phase modulation element **55** is not provided and

the fluorescence irradiation positions F21, F22, and F23 on the imaging surface 61 in the case where the phase modulation element 55 is provided, changes depending on the distance between the imaging surface 61 and the bright point of the fluorescence.

As shown in FIG. 10A, when the bright point of the fluorescence is at a position farthest from the imaging surface 61, that is, when the fluorescence is generated at a position most negative side, in the Y-axis direction, of the light sheet 11 included in the flow path 41, the fluorescence irradiation position F21 on the imaging surface 61 is displaced by a predetermined distance in the Z-axis positive direction with respect to the irradiation position F11 in the case where the phase modulation element 55 is not provided.

As shown in FIG. 10C, when the bright point of the fluorescence is at a position closest to the imaging surface 61, that is, when the fluorescence is generated at a position most positive side, in the Y-axis direction, of the light sheet 11 included in the flow path 41, the fluorescence irradiation position F23 on the imaging surface 61 is displaced by a predetermined distance in the Z-axis negative direction with respect to the irradiation position F13 in the case where the phase modulation element 55 is not provided.

As shown in FIG. 10B, when the bright point of the fluorescence is at an intermediate position between the bright point position shown in FIG. 10A and the bright point position shown in FIG. 10C in the Y-axis direction, that is, when the fluorescence is generated from an intermediate position, in the Y-axis direction, of the light sheet 11 included in the flow path 41, the fluorescence irradiation position F22 on the imaging surface 61 is displaced by a predetermined distance in the X-axis positive direction with respect to the irradiation position F12 in the case where the phase modulation element 55 is not provided.

When the fluorescence irradiation positions F11, F12, and F13 in the case where the phase modulation element 55 is provided are connected to the fluorescence irradiation positions F21, F22, and F23 in the case where the phase modulation element 55 is not provided, respectively, by straight lines, each straight line rotates in parallel to the X-Z plane in accordance with the distance between the imaging surface 61 and the bright point of the fluorescence.

The above-described optical effect causes shifting of the irradiation positions shown in FIG. 9. In FIG. 9, when lines L11, L12, and L13 parallel to the X-axis direction are set on the imaging surface 61, the distances in the Y-axis direction between the light sheet 11 and all the positions on the line L11 are constant, the distances in the Y-axis direction between the light sheet 11 and all the positions on the line L12 are constant, and the distance in the Y-axis direction between the light sheet 11 and all the positions on the line L13 are constant.

Therefore, all the fluorescences applied to the line L11 in the case where the phase modulation element 55 is not provided are shifted by the same distance in the same direction, due to the effect of the phase modulation element 55. All the fluorescences applied to the line L12 in the case where the phase modulation element 55 is not provided are shifted by the same distance in the same direction, due to the effect of the phase modulation element 55. All the fluorescences applied to the line L13 in the case where the phase modulation element 55 is not provided are shifted by the same distance in the same direction, due to the effect of the phase modulation element 55.

Therefore, the fluorescences applied to the positions P11, P12, and P13 in the irradiation area 111 in the case where the phase modulation element 55 is not provided, are applied to the positions P21, P22, and P23 in the irradiation area 121 in the case where the phase modulation element 55 is provided, respectively. A shift vector from the position P11 to the position P21, a shift vector from the position P12 to the position P22, and a shift vector from the position P13 to the position P23 are different from each other because the distances between the light sheet 11 and the lines L11, L12, and L13 in the Y-axis direction are different from each other, on the basis of the optical effect described with reference to FIGS. 10A to 10C. Therefore, the irradiation area 121 in the case where the phase modulation element 55 is provided is shifted while being deformed with respect to the irradiation area 111 in the case where the phase modulation element 55 is not provided. Thus, distortion occurs in the irradiation area 121.

The irradiation areas 122 and 123 shown in FIG. 8 are also shifted while being deformed with respect to the irradiation area 111 due to the optical effect of the phase modulation element 55, whereby distortion occurs in each of the irradiation areas 122 and 123. Since the positions, in the Y-axis direction, of the cross sections of the nucleus 12*a* corresponding to the irradiation areas 121, 122, and 123 are different from each other, distortions of the irradiation areas 121, 122, and 123 are different from each other. Therefore, if the images of the irradiation areas 121, 122, and 123 are used for generation of a three-dimensional image of the nucleus 12*a* without being corrected, the quality of the three-dimensional image may be degraded.

In Embodiment 1, the cross-sectional images of the nucleus 12*a* are subjected to correction for eliminating distortions, and a three-dimensional image of the nucleus 12*a* is generated by using the corrected cross-sectional images. Thus, the quality of the three-dimensional image can be improved. The distortion correction is performed by the processing section 81 shown in FIG. 1.

In this correction, the processing section 81 causes image elements forming the cross-sectional image to shift to positions at which displacement based on the PSF is corrected, respectively. For example, the processing section 81 performs a process of shifting an image element that is obtained by the imaging device 60 at each irradiation position in the irradiation area 121 shown in FIG. 9, to a position corresponding to each irradiation position in the irradiation area 111. Specifically, the processing section 81 performing a process of shifting the image elements obtained from the positions P21, P22, and P23 to the positions corresponding to the positions P11, P12, and P13. The processing section 81 performs processes similar to the above process, on the image elements obtained from all the irradiation positions in the irradiation area 121. Thus, distortion of the entire taken image can be appropriately corrected by performing the process of individually shifting the image elements.

In Embodiment 1, each image element is regarded as an image portion obtained for each pixel in the imaging device 60. Thus, when each image element to be corrected is set to an image element obtained from each pixel that is the minimum unit of imaging of the imaging device 60, distortion of the taken image can be corrected with high accuracy.

The image element regarded as a unit of distortion correction is not necessarily set for each pixel of the imaging device 60. An image portion obtained from a plurality of pixels included in a predetermined block unit may be regarded as an image element corresponding to a unit of distortion correction.

A specific process for distortion correction is as follows.

As shown in FIG. 11A, on the imaging surface 61 of the imaging device 60, lines of pixels arranged in the X-axis

direction are present. There are n lines of pixels from the uppermost pixel line PL1 to the lowermost pixel line PLn. As shown in FIG. **8**, the light sheet **11** is inclined, from the state parallel to the imaging surface **61**, by a predetermined angle in the direction parallel to the Y-Z plane. Therefore, the distances, in the Y-axis direction, between the light sheet **11** and the respective pixels on the pixel line PL1 are constant. Also regarding other pixel lines, the distances, in the Y-axis direction, between the light sheet **11** and the respective pixels on one pixel line are constant. The distance, in the Y-axis direction, between the light sheet **11** and each pixel line differs per pixel line.

Therefore, fluorescences generated from the positions, on the light sheet **11**, by the same distance apart from the imaging surface **61** in the Y-axis direction are incident on the respective pixels on the same pixel line. For example, in FIG. **9**, the fluorescences respectively incident on the two positions P**21** are incident on pixels on the same pixel line, the fluorescences respectively incident on the two positions P**22** are incident on pixels on the same pixel line, and the fluorescences respectively incident on the two positions P**23** are incident on pixels on the same pixel line.

The processing section **81** assigns, to each pixel line, as a correction vector, a vector that is opposite to each vector indicated by an arrow in FIG. **9**. Then, the processing section **81** causes image elements obtained from the respective pixels on one pixel line to be shifted by the correction vector that is set for this pixel line. The processing section **81** executes this process on all the pixel lines. Thereby, the respective image elements in the cross-sectional image are shifted to the positions in the case where the phase modulation element **55** is not provided. Thus, distortion of the cross-sectional image is corrected.

In this correction, the processing section **81** executes, for example, a process of mapping, in a memory, pixel values obtained from all the pixels on the imaging surface **61**, and shifting the mapped pixel values on the memory in accordance with the correction vectors. Alternatively, in this correction, the processing section **81** executes a process of causing registers to hold the pixel values obtained from the pixels on the respective pixel lines such that each register corresponds to one pixel line, and developing, on a memory, the pixel values held in the respective registers on the basis of the correction vectors assigned to the respective pixel lines.

In FIG. **11**A, the correction vectors assigned to the respective pixel lines are schematically shown as arrows. Specifically, the correction vectors for the respective pixel lines are represented on a table as shown in FIG. **11**B. An X correction value is a correction value in the X-axis direction, and is positive in the X-axis positive direction. A Z correction value is a correction value in the Z-axis direction, and is positive in the Z-axis positive direction. The processing section **81** causes the image elements, i.e., the pixel values, obtained from the respective pixels on the respective pixel lines to be shifted by the correction vectors thus defined.

As described above, the processing section **81** causes the respective image elements obtained from each pixel line that receives the fluorescence generated from the same position in the optical axis direction of the condensing optical system **50**, to be shifted in accordance with the direction and distance based on the bright point of the fluorescence, that is, in accordance with the correction vector, thereby eliminating distortion of the cross-sectional image of the nucleus **12***a*. Thus, distortion of the cross-sectional image can be easily and appropriately eliminated.

In Embodiment 1, as shown in FIGS. **10**A to **10**C, the direction of displacement of each irradiation position on the imaging surface **61** is set within the range of 180° from the Z-axis positive direction to the Z-axis negative direction. However, the range of the direction of displacement of the irradiation position is not limited thereto. However, if the range of the direction of displacement of the irradiation position exceeds 180°, fluorescences generated from the positions, on the light sheet **11**, having different distances from the imaging surface **61** may be simultaneously incident on some pixel lines. In this case, accuracy of distortion correction for the cross-sectional image is degraded. Therefore, the PSF of the phase modulation element **55** is preferably set such that the direction of displacement of the irradiation position on the imaging surface **61** is within the range of 180° from the Z-axis positive direction to the Z-axis negative direction.

Next, description is given of aspect-ratio correction and position adjustment when cross-sectional images having been subjected to distortion correction are superposed.

As shown in FIG. **8**, the nucleus **12***a* in the cell **12** flows in the flow path **41** of the flow cell **40** in the Z-axis positive direction. At this time, when the nucleus **12***a* passes through the light sheet **11**, fluorescence occurs from the cross section of the nucleus **12***a* to which the light sheet **11** is applied, and the generated fluorescence is applied to the imaging surface **61** of the imaging device **60**. In FIG. **8**, the nucleus **12***a* is shown as a sphere, for convenience. Assuming that the nucleus **12***a* is located at the positions **101** to **103** in the flow path **41** in order, fluorescences generated from the nucleus **12***a* located at the positions **101** to **103** are applied to the irradiation areas **121** to **123** on the imaging surface **61**, respectively. These irradiation areas **121** to **123** are substantially corrected, through the aforementioned distortion correction, to the irradiation areas **111** to **113** in the case where the phase modulation element **55** is not provided.

Since the light sheet **11** is inclined with respect to the Z-axis direction, the length, in the Z-axis direction, of each irradiation area on the imaging surface **61** is shorter than the length, in the first direction D1, of the corresponding cross section to which the light sheet **11** is applied. Specifically, the length, in the Z-axis direction, of the irradiation area on the imaging surface **61** has a value obtained by multiplying the length, in the first direction D1, of the corresponding cross section to which the light sheet **11** is applied, by cos θ. Therefore, by multiplying the length of the irradiation area in the Z-axis direction by 1/cos θ, the distortion-corrected irradiation area can be corrected to have an appropriate aspect ratio in which the actual cross-sectional shape is reflected.

It is assumed that, when the nucleus **12***a* is at the position **102**, the light sheet **11** is applied to the center of the nucleus **12***a*. At this time, assuming that the position of the nucleus **12***a* on the Z-axis is 0, the position of the irradiation area **112** on the Z-axis is also 0. However, when the nucleus **12***a* is at a position different from the position **102**, the light sheet **11** is not applied to the center of the nucleus **12***a*. In this case, displacement occurs between the position of the nucleus **12***a* and the position of the distortion-corrected irradiation area.

It is assumed that the position, on the Z-axis, of the nucleus **12***a* at the position **103** is x1, and the position, on the Z-axis, of the irradiation area **113** is x2. That is, it is assumed that the amount of movement of the nucleus **12***a* in the flow path **41** of the flow cell **40** is x1, and the amount of movement of the image of the nucleus **12***a* on the imaging surface **61** is x2. When the angle of inclination of the light

sheet **11** with respect to the sample flow direction is θ, x**2** is calculated according to the following formula (1).

$$x2=x1(1-\sin^2\theta) \quad (1)$$

It is assumed that, as shown in FIG. **12**A, distortion-corrected images **201** to **203** are obtained on the basis of the nucleus **12***a* at the positions **102** to **103**, respectively. In the images **201** to **203**, the distortion-corrected irradiation areas **112** to **114** corresponding to the nucleus **12***a* are included.

In order to superpose the images **201** to **203**, for example, a timing at which the image **201** in which the position of the irradiation area on the Z-axis is 0, is set as a reference time. The amount of movement x**11** of the nucleus **12***a* when an imaging interval Δt has passed from the reference time can be calculated by multiplying Δt by the sample flow speed. At this time, the position x**21** of the irradiation area **113** can be obtained by substituting x**11** for x**1** in the above formula (1). Likewise, the amount of movement x**12** of the nucleus **12***a* when an imaging interval 2Δt has passed from the reference time can be calculated by multiplying 2Δt by the sample flow speed. At this time, the position x**22** of the irradiation area **114** can be obtained by substituting x**12** for x**1** in the above formula (1). Then, in the image **202**, the irradiation area **113** is shifted by x**21** in the direction approaching the position 0 on the Z-axis. Likewise, also in the image **203**, the irradiation area **114** is shifted by x**22** in the direction approaching the position 0 on the Z-axis.

The images taken by the imaging device **60** are successively stored in the storage section **82**. The processing section **81** of the information processing unit **10***b* groups all the distortion-corrected images from the first cross-sectional image to the last cross-sectional image that have been obtained from one nucleus **12***a*, among a plurality of images stored in the storage section **82**. In grouping the distortion-corrected images, a distortion-corrected image previous to the first distortion-corrected image of the nucleus **12***a* and a distortion-corrected image subsequent to the last distortion-corrected image of the nucleus **12***a* may be included in the group. The interval of imaging by the imaging device **60** is determined on the basis of the speed of the sample that flows in the flow path **41**, the size of the cell, the thickness of the light sheet **11** in the second direction D**2**, etc., such that the number of images taken from one cell is about 2 to 100.

For example, as shown on the left side in FIG. **12**B, the processing section **81** groups the distortion-corrected images **201** to **207**. The processing section **81** subjects the grouped distortion-corrected images **201** to **207** to correction of the aspect ratios of the irradiation areas on the respective images as described above. Then, the processing section **81** subjects the aspect-ratio-corrected images **201** to **207** to position adjustment for the irradiation areas on the respective images as described above. Thus, as shown on the right side in FIG. **12**B, images **211** to **217** are obtained which have been subjected to the above-described distortion correction, aspect-ratio correction, and adjustment of center positions of the irradiation areas.

Then, the processing section **81** superposes the images **211** to **217** which have been subjected to the aspect-ratio correction and the position adjustment, thereby generating an appropriate three-dimensional image of one nucleus **12***a*. Thus, a high-quality three-dimensional image can be obtained.

The aspect-ratio correction and the position adjustment performed for generating a three-dimensional image are not limited to the above-described methods, and the following methods may be adopted, for example.

A sample containing spherical particles such as fluorescence beads is caused to flow in the flow cell **40**, and images of each particle are taken by the imaging device **60**. In each of the taken images, a center coordinate of the particle cross-section in the flow direction is obtained. Then, to what extent each image should be shifted to make the center coordinates of the particle cross-sections coincide with each other, is calculated as a parameter for correction of displacement. Further, in any one of the images, to what extent the image should be extended in the flow direction to make the particle cross-section completely round, is calculated as a parameter for correction of the aspect ratio. Two parameters thus obtained are stored in the storage section **82**.

When a three-dimensional image is generated on the basis of an actual sample, the processing section **81** subjects each distortion-corrected image to aspect-ratio correction and position adjustment, by using the two parameters stored in the storage section **82**. Then, the processing section **81** superposes the images that have been subjected to the aspect-ratio correction and the position adjustment, thereby generating a three-dimensional image. In this case, the aspect-ratio correction and the position adjustment can be performed on the basis of the actual states of the optical system and the like in the cell imaging apparatus **10**, whereby a high-definition three-dimensional image can be generated. The two parameters may be obtained based on one bead, but preferably are obtained by averaging parameters obtained based on a plurality of beads.

Next, the relationship between inclination of the light sheet **11** and imaging accuracy is described.

As shown in FIGS. **13**A and **13**B, the light sheet **11** has a predetermined thickness in the second direction D**2** according to the rotational position of the optical lens **31**. When the light sheet **11** is applied to the center of the nucleus **12***a*, the thickness of the light sheet **11** in the optical axis direction of the condensing optical system **50**, i.e., in the Y-axis direction, is equal to a width d. In this case, fluorescence is generated not only from the center of the nucleus **12***a* but also from a portion of the nucleus **12***a* included in the range of the width d. Therefore, when imaging of the center of the nucleus **12***a* is performed, the fluorescence generated from the portion, of the nucleus **12***a*, other than the center portion becomes a noise component. Such a noise component causes background noise of the taken image, and therefore is preferably as small as possible.

As shown in FIG. **13**A, the width d increases as the inclination angle θ of the sheet surface **11***a* with respect to the Z-axis approaches 90°. On the other hand, as shown in FIG. **13**B, the width d decreases as the inclination angle θ of the sheet surface **11***a* with respect to the Z-axis approaches 0°. Therefore, in order to reduce the noise component, the angle θ is preferably as small as possible. However, as described above, when the angle θ is 0°, a plurality of different cross-sectional images cannot be obtained. Therefore, the angle θ needs to be greater than at least 0°.

Next, description is given of the conditions for the angle θ that allows obtainment of all the cross sections while reducing the noise component.

As shown in FIG. **14**A, it is assumed that the diameter of the nucleus **12***a* is A**1**, and the size of an imaging field-of-view in the flow path **41**, i.e., the width, in the Z-axis direction, of the flow path **41** that can be imaged, is B**1**. In order to obtain all the cross sections of the nucleus **12***a* while reducing the angle θ for reduction of the noise component, the light sheet **11** needs to cover one end, of the nucleus **12***a*, positioned at an upper end of the imaging field-of-view and the other end, of the nucleus **12***a*, positioned at a lower end

of the imaging field-of-view. Therefore, an optimum angle θ is calculated from the following formula (2).

$$\tan\theta = A1/B1 \quad (2)$$

When the angle θ is set so as to satisfy the formula (2), a high-definition image with reduced background noise can be taken while obtaining all the cross-sectional images of the nucleus **12***a*.

Next, description is given of a process of applying the optimum angle θ as described above to the cell imaging apparatus **10**.

As shown in FIG. **14**B, in step S**1**, the processing section **81** receives a numerical value that is inputted by a user through the input section **84**, and sets, on the rotation controller **70**, the received numerical value as an average size of an imaging target, i.e., as an average width of the imaging target in the optical axis direction of the condensing optical system **50**. In Embodiment 1, the user inputs an average diameter of the nucleus **12***a*.

In step S**1**, the processing section **81** may cause the display section **83** to display a list of imaging targets, and may receive an imaging target that is selected by the user through the input section **84**. In this case, the processing section **81** reads, from a mapping table stored in the storage section **82** in advance, the size corresponding to the imaging target received from the user, and sets the read size on the rotation controller **70**. Alternatively, in step S**1**, the processing section **81** may calculate the size of an imaging target on the basis of an image taken by the imaging device **60** in advance, and may set the calculated size on the rotation controller **70**.

In step S**2**, the processing section **81** receives the size of the field of view that is inputted by the user through the input section **84**, and sets the received size of the field of view on the rotation controller **70**. The size of the field of view changes depending on the magnification of the object lens **51** in the condensing optical system **50**, the number of pixels of the imaging device **60**, etc.

In step S**2**, the processing section **81** may cause the display section **83** to display a list of object lenses **51** and a list of imaging devices **60**, and may receive an object lens **51** and an imaging device **60** that are selected by the user through the input section **84**. In this case, the processing section **81** may read, from a mapping table stored in the storage section **82** in advance, the magnification of the object lens **51** received from the user and the number of pixels of the imaging device **60** received from the user. Then, the processing section **81** may calculate the size of the field of view on the basis of the magnification and the number of pixels, which have been read, and set the calculated size of the field of view on the rotation controller **70**.

In step S**3**, the rotation controller **70** puts the size of the imaging target and the size of the field of view, which have been set by the processing section **81**, into the above formula (2), thereby calculating the inclination angle θ of the light sheet **11**. The processing section **81** may calculate the inclination angle θ of the light sheet **11** and transmit the calculated angle θ to the rotation controller **70**.

In step S**4**, the rotation controller **70** causes the rotation mechanism section **32** to rotate the optical lens **31** such that the inclination of the light sheet **11** becomes the angle θ that is calculated in step S**3**. Thus, the inclination of the light sheet **11** is set such that all the cross-sectional images of the imaging target can be obtained and a high-definition image with reduced background noise can be taken.

The rotation controller **70** is not necessarily provided, and may be omitted. In this case, for example, an operator manually rotates the rotation mechanism section **32** such that the angle of the optical lens **31** becomes θ. Instead of the rotation mechanism section **32**, a plurality of holders each having an optical lens **31** fixed thereto may be prepared so as to correspond to a plurality of angles of the optical lens **31**. In this case, when an angle θ is calculated, a holder corresponding to the calculated angle θ is selected, and the selected holder is placed in the apparatus, whereby the angle of the optical lens **31** is changed. Arrangement of the holder may be performed manually or automatically.

Next, a process of generating a three-dimensional image is described.

As shown in FIG. **15**, in step S**11**, the user causes the light source **20** to emit light, thereby applying the light sheet **11** to a sample. The light source **20** may be connected to the interface **85**. In this case, in step S**11**, the processing section **81** controls the light source **20** to apply the light sheet **11** to the sample.

In step S**12**, the user causes the sample containing a plurality of cells to flow in the flow path **41** of the flow cell **40**. The sample is prepared such that the plurality of cells simultaneously cross the light sheet **11**. The imaging unit **10***a* may include: a storage section for storing therein the prepared sample; and a transfer section for transferring the sample stored in the storage section to the flow cell **40**. In this case, in step S**11**, the processing section **81** controls the transfer section of the cell imaging apparatus **10** so that the sample stored in the storage section flows in the flow cell **40**.

In step S**13**, the processing section **81** causes the imaging device **60** to take images of fluorescences generated from nuclei **12***a* in the plurality of cells **12**. Specifically, the images of the fluorescences are sequentially taken on the basis of the frame rate of the imaging device **60**, and the taken images are sequentially stored in the storage section **82**. In step S**14**, the processing section **81** generates an image including three-dimensional images of the plurality of cells, on the basis of the plurality of images taken by the imaging device **60**. In step S**14**, a process shown in FIG. **16** is performed.

In step S**21**, the processing section **81** subjects the plurality of images taken by the imaging device **60** to distortion correction as described with reference to FIGS. **11**A and **11**B. Further, in steps S**22** and S**23**, the processing section **81** subjects the plurality of distortion-corrected images to aspect-ratio correction and position adjustment as described with reference to FIG. **8** and FIG. **12**A. That is, regarding a plurality of distortion-corrected images obtained from one nucleus **12***a*, the processing section **81** calculates the amount of movement of the nucleus **12***a* on the imaging surface **61** on the basis of the above formula (1), thereby performing aspect-ratio correction. In step S**24**, the processing section **81** performs superposition of the images as described with reference to FIG. **12**B, on the basis of the calculated amount of movement, and the aspect-ratio-corrected images. Thus, the processing section **81** generates an image including the three-dimensional images of the plurality of nuclei **12***a*.

If an aggregate of a plurality of cells crosses the light sheet **11**, for example, a three-dimensional image as shown in FIG. **17** may be obtained through the processes shown in FIG. **15** and FIG. **16**. In FIG. **17**, an image including three-dimensional images of four nuclei **12***a* is obtained.

Embodiment 2

In the condensing optical system **50** according to Embodiment 2, the phase modulation element **55** is replaced with a phase modulation element that forms a double-helix PSF.

The "double-helix PSF" is a kind of a spiral point spread function, and is a point spread function that allows light generated from one bright point to be imaged onto two focal points. In Embodiment 2, the phase modulation pattern shown in FIG. **5**A is replaced with a phase modulation pattern that forms a double-helix PSF. Other components of Embodiment 2 are identical to those of Embodiment 1.

As shown in FIG. **18**, fluorescences generated from points at different positions in the Y-axis direction are imaged onto two focal points on the imaging surface **61** of the imaging device **60**. At this time, the two focal points rotate on the imaging surface **61** in accordance with the positions of the bright points of the fluorescences in the Y-axis direction. That is, an angle formed by a reference line and a line connecting the two focal points changes on the imaging surface **61** in accordance with the positions of the bright points of the fluorescences in the Y-axis direction.

As shown in FIG. **19**, in a case where the phase modulation element **55** is not provided, fluorescence generated from one bright point at a cell cross section is applied to the imaging surface **61** in the irradiation area **111**. On the other hand, when the phase modulation element **55** that forms a double-helix PSF is provided, as shown in FIG. **19**, fluorescence generated from one bright point at a cell cross section is applied to two irradiation areas **121**. In this case, the fluorescence, which has been applied to an irradiation position P**41** in the irradiation area **111** when the phase modulation element **55** is not provided, is split into two beams to be applied to irradiation positions P**42** in the two irradiation areas **121**.

Therefore, when the phase modulation element **55** that forms a double-helix PSF is used, the image elements obtained from the two irradiation areas **121** can be shifted to the irradiation area **111** by using, as correction vectors, vectors that are opposite to the vectors respectively directed to the two irradiation areas **121** from the irradiation area **111**, i.e., vectors indicated by solid lines in FIG. **19**.

In the distortion correction process, the processing section **81** searches the pixels on the imaging surface **61**, to which the fluorescence is applied, for two pixels to be paired. For example, the processing section **81** selects pixel lines, one by one, from the uppermost pixel line. In each selected pixel line, the processing section **81** specifies a pixel to which the fluorescence is applied. Then, the processing section **81** specifies a pixel in another pixel line, which is to be paired with the above specified pixel, on the basis of the positional relationship of the two focal points shown in FIG. **18**, and if the fluorescence is applied to this pixel, the processing section **81** obtains these two pixels as pixels to be paired. As shown in FIG. **18**, the positional relationship between the two focal points differs per selected pixel line, that is, differs according to the distance between the imaging surface and the bright point of the fluorescence. Therefore, each pixel in each pixel line is associated with another pixel in another pixel line to be paired therewith due to the effect of the double-helix PSF. On the basis of the association relationship, the processing section **81** obtains two images to which the fluorescence is applied, as two pixels to be paired. Thus, the processing section **81** obtains pixels to be paired, from all the pixels in all the pixel lines.

Thereafter, the processing section **81** superposes the image elements obtained from the pair of pixels onto an intermediate pixel position between these pixels. The processing section **81** performs this process for all the pairs of pixels. Thus, all the pairs of image elements obtained from the cell cross section are superposed on one another. Thus, the processing section **81** obtains the distortion-corrected cross-sectional image of the cell. The process after the distortion correction is the same as that of Embodiment 1.

Also by the configuration of Embodiment 2, a distortion-corrected cross-sectional image can be obtained. Therefore, a high-quality cell image can be obtained as in Embodiment 1.

In the configuration of Embodiment 2, however, since the fluorescence generated from one bright point is split into two beams to be applied to the imaging surface **61**, fluorescences generated from the cross section of the cell needs to be separated on the imaging surface **61** so that pairs of image elements obtained from the pixels on the imaging surface **61** can be specified. Embodiment 2 is applicable to a cell that allows separation of fluorescences in the above-described manner. For example, when the size of each cell is small and the density of cells flowing in the flow cell **40** is low, fluorescences at the bright points generated from the cross section of the cell are separated on the imaging surface **61** so that the pairs can be specified. The configuration of Embodiment 2 is applicable to such a case.

In contrast to Embodiment 2, since the phase modulation element **55** that forms the single-helix PSF is used in Embodiment 1, fluorescences generated from the portions at the cell cross section are not separated. Therefore, for any cell, distortion that occurs in a taken image can be smoothly corrected.

For only one of the two irradiation areas **121** shown in FIG. **19**, the image element thereof may be shifted by the correction vector to obtain a distortion-corrected cross-sectional image. In this case, however, the distortion-corrected cross-sectional image is darker than that obtain in the case where the image elements of the two irradiation areas **121** are superposed. In Embodiment 2, a bright cross-sectional image can be obtained by superposing the paired image elements.

As for the phase modulation element **55**, a phase modulation element that forms a multi-helix PSF equal to or more than triple-helix may be used. The "multi-helix PSF" is a kind of a spiral point spread function, and is a point spread function that allows light generated from one bright point to be imaged onto a plurality of focal points. Also in this case, paired image elements may be superposed on an intermediate position between the image elements. Thus, a bright cross-sectional image can be obtained. For example, when a phase modulation element that forms a triple or more helix PSF is used as the phase modulation element **55**, a set of three image elements may be interposed on an intermediate position among the three image elements, i.e., a center-of-gravity position among the three image elements.

While in Embodiments 1 and 2, light to be imaged by the imaging device **60** is fluorescence, light to be imaged by the imaging device **60** may be light that is generated on the side of the flow cell **40** from a cell as an imaging target, for example, side scattered light.

While in Embodiments 1 and 2, the flow cell **40** has a square outer shape as viewed in the Z-axis direction, the flow cell **40** may have a circular outer shape as viewed in the Z-axis direction. For example, when the flow cell **40** is formed in a columnar shape, the flow cell **40** has a circular outer shape as viewed in the Z-axis direction, and the outer side surface of the flow cell **40** is a curved surface. When the flow cell **40** is formed in a columnar shape, the light sheet **11** is perpendicular to the tangential plane of the outer side surface of the flow cell **40**. In this case, the light sheet **11** incident on the flow cell **40** is inhibited from being deflected by the outer side surface, whereby the shape of the light

sheet **11** applied to the cell **12** is less likely to be deformed. Accordingly, the imaging device **60** is allowed to take a high-definition image.

Modifications

In Embodiments 1 and 2, description has been given of the configuration in which a plurality of cells are simultaneously imaged. However, it is possible to extract an invention regarding distortion correction for a taken image. In this case, a plurality of cells are not necessarily imaged simultaneously. A sample may be caused to flow in the flow cell **40** such that only one cell crosses the light sheet **11**.

Particles to be imaged are not limited to cells, and may be particles other than cells. For examples, particles to be imaged may be organism-derived particles other than cells, light-transmitting particles such as fluorescence beads, or the like. That is, any particles may be used as long as the particles have light translucency and generate light to the outside of the flow cell when being irradiated with light. Further, fluorescence images of HER2 gene and CEP17 as a centromere region of chromosome **17** may be obtained as well as the fluorescence image of the nucleus **12***a*. Besides, fluorescence images of other portions in a cell, such as other genes, nucleus acids, cytoplasm, protein, organelle, etc., may be obtained.

A particle imaging apparatus according to this modification may have the same configuration as the configurations of the cell imaging apparatuses **10** according to Embodiments 1 and 2. A particle imaging method according to this modification may be the same as the processes shown in FIGS. **15** and **16** of Embodiments 1 and 2. In this modification, distortion correction for taken images may be performed in the same manner as step S**21** of Embodiments 1 and 2. In addition, the processes in steps S**22** and S**23** in FIG. **16** are also performed in the same manner.

When HER2 gene and CEP17 are imaging targets as well as the nucleus **12***a*, fluorescences generated from the HER2 gene and the CEP17 are projected as bright points in the irradiation area of the nucleus **12***a*, and therefore, the HER2 gene and the CEP17 are also included in the cross-sectional image of the nucleus **12***a*. Further, according to the distortion correction, aspect-ratio correction, and position adjustment as described above, the HER2 gene and the CEP17 are also subjected to distortion correction, aspect-ratio correction, and position adjustment. Therefore, in this modification, the bright points of the HER2 gene and the CEP17 are included in a three-dimensional image, of the nucleus **12***a*, formed by superposing the cross-sectional images of the nucleus **12***a*. Thus, according to this modification, regarding not only a portion having a certain size, such as the nucleus **12***a*, but also fine portions such as the HER2 gene and the CEP17, a three-dimensional image in which three-dimensional distribution states of these portions are reflected can be obtained on the basis of a plurality of images taken by the imaging device **60**.

The element (**55**) for extending the depth of focus is not limited to a phase modulation element, and a variable focal point lens may be used to extend the depth of focus.

What is claimed is:

1. A cell imaging method comprising:

forming a light sheet having a flat sheet surface in a flow path of a flow cell;

flowing a measurement sample containing a plurality of cells in the flow cell and through the light sheet;

directing light generated from the plurality of cells passing through the light sheet to an imaging device via an optical system, the optical system including an objective lens and an element configured to extend a depth of focus of the imaging device, the element located between the objective lens and the imaging device in a light path; and

taking images of the plurality of cells with the imaging device,

wherein the light sheet is formed such that the flat sheet surface is not perpendicular or parallel to a flow direction of the sample and the distance between the flat sheet surface and the optical system is not constant along the flow direction; and

correcting distortion of the taken image by:

shifting first image elements corresponding to a first line of pixels of the imaging device in a first direction and by a first distance; and

shifting second image elements corresponding to a second line of pixels of the imaging device in a second direction and by a second distance,

wherein the first line of pixels are in a same distance from the light sheet and the second line of pixels are in a same distance from the light sheet, and

the element is a phase modulation element configured to modulate a phase of light from the plurality of cells passing through the light sheet according to a single-helix point spread function.

2. The cell imaging method of claim **1**, wherein the plurality of cells are caused to simultaneously pass through the light sheet, and lights generated from the plurality of cells are received by the imaging device.

3. The cell imaging method of claim **1**, wherein the point spread function is a spiral point spread function.

4. The cell imaging method of claim **1**, wherein the distortion is caused by the phase modulation element.

5. The cell imaging method of claim **4**, wherein in the correcting of the distortion of the taken image, the first image elements and the second image elements are each shifted to a position at which displacement thereof based on the point spread function is corrected.

6. The cell imaging method of claim **5**, wherein the first distance is between the light sheet and a position, on an imaging surface, at which the first image elements are obtained.

7. The cell imaging method of claim **6**, wherein the first direction and the first distance are based on the first distance between the light sheet and the position, on the imaging surface, at which the image elements are obtained.

8. The cell imaging method of claim **5**, wherein each image element of the first image elements is an image element obtained pixel by pixel.

9. The cell imaging method of claim **1**, further comprising:

taking a plurality of images each including a plurality of cells; and

generating an image including three-dimensional images of the plurality of cells on the basis of the plurality of taken images.

10. The cell imaging method of claim **9**, wherein:

the generating of the image includes correcting a position of the image of each cell at the imaging surface, and

in the correcting of the position, an amount of shifting of the image of the cell on the imaging surface is calculated on the basis of, at least, an amount of movement of the cell in the flow cell, and an angle of the light sheet with respect to a flow direction of the sample, and

the three-dimensional image of the cell is generated on the basis of the calculated amount of shifting, and a series of the taken images obtained along with movement of the cell.

11. The cell imaging method of claim **9**, wherein:

the generating of the image includes correcting a size of the image of each cell on the imaging surface, and

in the correcting of the size, the size of the taken image of the cell is corrected on the basis of an angle of the light sheet with respect to a flow direction of the sample, and

the three-dimensional image of the cell is generated on the basis of the size-corrected image.

12. A cell imaging apparatus comprising:

a flow cell configured to cause a sample containing a plurality of cells to flow therein;

a light source;

an irradiation optical system configured to form, with respect to the flow cell, a light sheet having a flat sheet surface from light emitted from the light source;

a condensing optical system having an objective lens and an element configured to extend a depth of focus, the condensing optical system being configured to condense light generated from the plurality of cells flowing in the flow cell;

an imaging device configured to receive light generated from the plurality of cells and condensed by the condensing optical system, and take images of the plurality of cells,

wherein the light sheet is such that the flat sheet surface is not perpendicular or parallel to a flow direction of the sample and a distance between the flat sheet surface and the condensing optical system is not constant along the flow direction, and

wherein the element is a phase modulation element configured to modulate a phase of light from the plurality of cells passing through the light sheet according to a single-helix point spread function; and

at least one processor configured to correct distortion of at least one of the images by:

shifting first image elements corresponding to a first line of pixels of the imaging device in a first direction and by a first distance; and

shifting second image elements corresponding to a second line of pixels of the imaging device in a second direction and by a second distance,

wherein the first line of pixels are in a same distance from the light sheet and the second line of pixels are in a same distance from the light sheet.

13. The cell imaging apparatus of claim **12**, wherein the phase modulation element forms a spiral point spread function at an imaging surface of the imaging device.

14. The cell imaging apparatus of claim **13**, wherein the spiral point spread function is a single-helix point spread function.

15. A particle imaging method comprising:

forming a light sheet having a flat sheet surface with respect to a flow cell facing to the flat sheet surface;

taking an image of light generated from a particle that flows in the flow cell, via a phase modulation element configured to modulate a point spread function; and

correcting distortion of the taken image, the distortion being caused by the phase modulation element, the correcting distortion comprising:

shifting first image elements corresponding to a first line of pixels of the imaging device in a first direction and by a first distance; and

shifting second image elements corresponding to a second line of pixels of the imaging device in a second direction and by a second distance,

wherein the first line of pixels are in a same distance from the light sheet and the second line of pixels are in a same distance from the light sheet, and

wherein the point spread function is a single-helix point spread function.

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